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TITLE:
Nebraska Prostate Cancer Research Program

PRINCIPAL INVESTIGATOR:
Ming-Fong Lin, Ph.D.

CONTRACTING ORGANIZATION:
University of Nebraska
Omaha, NE 69198-6810

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14. ABSTRACT
Prostate cancer (PCa) is one of the major cancers threatening US males' life and has a high incidence in American African males. For improving PCa treatments, better understanding the basic mechanism of this cancer is needed, which would depend on the training of more PCa researchers. The **purpose** of this grant is to train HBCU undergraduates with science major in PCa research. The **scope** of this training grant: Dr. Ming-Fong Lin of the University of Nebraska Medical Center (UNMC) and Dr. Shafiq Khan of Clark Atlanta University (CAU) have jointly instituted this training grant between UNMC and CAU for training CAU students in PCa research. The **major finding** and **up-to-date** report: Drs. Lin and Khan had identified interested undergraduates at CAU and instituted a summer research training program at UNMC where Dr. Lin recruited PCa scientist as mentors and CAU students learned translational research in labs. Students had spent the majority of their time working at the bench on a research project. They also participated in seminar series that were specifically organized for summer undergraduate students and introduced them to different areas of scientific investigation and advanced technological tools used in scientific discovery. During the academic year, students continued their training at CAU, which is arranged by Dr. Khan. This continuous training prepares students for a graduate career in biomedical sciences or medical school.

15. SUBJECT TERMS
Prostate Cancer, Training Grant, HBCU students, Biomedical Sciences, Graduate school, Medical school

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Annual Summary

1. **INTRODUCTION:**

The subject of this training grant is to train potential prostate cancer (PCa) researchers via a collaborative effort between University of Nebraska Medical Center (UNMC) at Omaha, Nebraska, and Clark Atlanta University (CAU) at Atlanta, Georgia. The conception of this Program is based on the interactions and collaborations between Dr. Ming-Fong Lin, the PI and a faculty mentor at UNMC, and Dr. Shafiq Khan, a faculty mentor at CAU, since March 2004 with their first joint peer-reviewed publication in 2008 (1). **The purpose** of this proposal is to train undergraduate HBCU students from CAU to gain hand-on experience in performing research on PCa, a high incidence cancer in Africa American males (2), in UNMC, a PCa research-intensive environment. **The scope** of training grant is to train undergraduate students from CAU, a traditional HBCU, to gain hand-on experience in PCa research at UNMC Nebraska Prostate Cancer Research Program (NPCRP). These students will receive training not only in the lab but also in the class room in the format of seminars and visiting biotechnology companies. **The goal** is to encourage and to prepare HBCU students for academic career, i.e., they will either enter graduate school or medical school with training in, and understand of, PCa research or enter medical school. This will increase the number of PCa researcher at both basic science and clinical science levels.

2. **KEYWORDS:**

CAU, Clark Atlanta University; CDMRP, Congressionally Directed Medical Research Programs; DOD, Department of Defense; HBCU, Historically Black Colleges and Universities; INBRE, Idea Networks of Biomedical Research Excellence; NPCRP, Nebraska Prostate Cancer Research Program; PCa, Prostate cancer; UNMC, University of Nebraska Medical Center; UNMC SURP; UNMC Summer Undergraduate Research Program;

3. **OVERALL PROJECT SUMMARY:**

This report serves as the annual report of the funding support from CDMRP between 09/15/2013 – 09/14/2014. Our NPCRP Program was previously awarded with a training grant between 5/2010 – 4/2013 with no-cost extension to 4/2014 (PC094594). For the continuation of training purpose with many inquiries from students, we obtained the approval from UNMC to initiate student recruitment process prior to the official funding of the current award. Under current award, we recruited 4 CAU undergraduate students in Spring 2013 for their training in Summer 2013. These 4 students had gained hand-on experience in PCa research and made significant accomplishments. This section is in direct alignment with respect to each task outlined in the approved SOW. There were no technical or unexpected difficulties encountered and/or any deviations from the original Statement of Work. Per Guidelines, our training and research accomplishments following each task outlined in the approved Statement of Work are listed as follows:

(**Task 1 - 4 for 2014 Annual Report**)

Task 1: Announcement of the Year 1 Research Program (months 1-3)

Done. (a) Per approved SOW, in January 2013, Drs. Khan and Odero-Marah at CAU and Drs. Lin and Chaney at UNMC started to up-date the flier announcing the opportunity of conducting PCa research at UNMC. The wording in the flier was finalized by the end of January, and Drs. Khan, Odero-Marah and also other PCa faculty members at CAU Cancer

Center immediately announced the opportunity by distributing the flier and verbal announcements in their classes and also campus-wide posters by Dr. Khan's office in Feb 2013.
(b) The announcements included the criteria of eligibility, the requirement of documents, and the due date of application.
(c) Drs. Khan and Odero-Marah and Dr. Khan's office Ms. Bakari, the office manager, prepared all the necessary paper works and answered to all questions related to this opportunity. The final due date was set as by March 31, 2013.

Task 2: Selection of Trainees (month 4-6)
Done. (a) Dr. Khan's staff members went through all application files to ensure all application documents were complete and in place.
(b) Drs. Chaney and Lin visited CAU on March 17, 2013, met with Drs. Khan and Odero-Marah and discussed for student recruitment processes, and also met with 4 eligible student candidates on March 19-20, 2013. Drs. Odero-Marah sent all applicants' information to Dr. Chaney in the early April of 2013, after the due date of application. This year, we had 12 students who filed their applications.
(c) With the inputs of Dr. Odero-Marah, Drs. Chaney and Lin discussed all the applicants' qualification. Drs. Chaney and Odero-Marah also discussed and matched the candidate's interest with the research lab.
(d) Four successful applicants were identified and notified by e-mails from Dr. Chaney and also contacted by a secretary at CAU Cancer Center. The students were given a due date for replying of their acceptance.
(e) Dr. Chaney also coordinated the Housing issue at UNMC, and Ms. Karen at Department of Biochemistry and Molecular Biology, UNMC, mailed the first batch of documents including Housing information to students for their attention. With the support of Dr. Turpen, the NPCRP students continuously interacted with the INBRE program for student training, including attending the seminars and site visiting to various research facilities and biotech companies. We also registered those students for the UNMC SURP program.
(f) All students arrived on Monday of May 27, 2013. Dr. Chaney met students at the Omaha Eppley airport and drove them to the dorm. Drs. Chaney and Lin welcome them and had dinner with all students.

Task 3: Summer Research (months 7-9)
Done. (a) All students started their orientation on Tuesday of May 28 and continued on Wednesday of May 29. The INBRE Program had a Welcome Barbeque reception in the evening of May 29 and CAU students were also invited. In the afternoon of May 30, students reported to the matched lab and began their summer research in PCa. Dr. Chaney attended students' Monday seminar and met with students at least weekly. Dr. Chaney also gave students rides for grocery shopping weekly or whenever there was a need.
(b) Whenever possibly, Dr. Lin visited students in the labs to make sure everything was going well. He also met with all students after four weeks of training in research labs to discuss any potential issues or suggestions for improvement.
(c) Drs. Lin and Chaney invited Dr. Khan visiting UNMC, meeting with CAU trainees and faculty mentors and discussing with their research progress. Dr. Khan visited UNMC on July 7, 2013 (**Appendix #1**). He met with CAU students and had a lunch-meeting together to

learn their progresses. Drs. Lin and Chaney and also other mentors Drs. Batra, Cheng, Datta and Mehta all attended the lunch-meeting. After the lunch, Dr. Khan had a private meeting with students discussing any potential problem during their stays. Subsequently, Dr. Khan met with Drs. Lin and Chaney for an executive meeting discussing students' issues. Overall, all CAU students were very happy, enjoyed their stays and had obtained the hand-on experience in their research projects. They were very pleased with the research environment without any complain or suggestion.

Dr. Khan gave a scientific presentation on his research projects and also a brief introduction of research environment at the CAU to the UNMC community (**Appendix #1**). His presentation was well received by audience, including faculty members, post-doctoral fellows and graduate students. Dr. Khan also had meetings with mentors and other faculty members with similar research interests in PCa to discuss the potential of collaborations (**Appendix #2**).

(d) In the last week of training, all students prepared their posters. Due to their departure on Saturday of August 3, they were unable to present their posters in the UNMC Undergraduate Summer Research Program in August 9. Nevertheless, they learned how to present their data by preparing scientific posters, and these posters would also allow them to give presentations in future meetings. All the posters are attached (**Appendices #3-6**). Prior to their departure, Drs. Lin and Chaney met with students and had a farewell lunch as Dr. Lin's guests prior to their return to CAU.

Task 4: Evaluation of the Program (months 10-12)

Done. (a) Prior to their departure, all CAU students met with Drs. Chaney and Lin and other faculty mentors for a final lunch-meeting. We discussed any problem that occurred during their stays and any suggestion that will improve the training in future. The students were also asked to prepare the anonymous evaluations. From these evaluation and comments, we understand the issues raised by the students. We will implement these suggestions and improve our processes for a better training program.

Dr. Chaney met with Dr. Turpen and discussed the results of NPCRP training program during their INBRE Retreat. We appreciated very much for the strong supports from Dr. Turpen and the INBRE program to NPCRP. With such a cooperating effort, the CAU students could expose to different technologies applicable toward research and career development and also meeting with student peers for social events. We expect the continuing interactions in the up-coming years. Drs. Chaney and Lin met and discussed the potential improvement for the Training Program per students' comments.

(b) Drs. Khan and Odero-Marah and Ms. Bakari met with all CAU students at CAU and discussed the evaluation and concerns at the beginning of Fall semester (**Appendix #7**). All CAU students in general were very impressed and happy with the opportunity of training at UNMC, Omaha, NE. There was no other issue raised. Dr Khan then phoned Dr. Lin regarding the conclusion of meeting with students' evaluation and expressed students' positive attitude toward research experience in NPCRP at UNMC.

(c) Drs. Lin and Chaney met and discussed with mentors for inputs of improving the training quality for the following year. They also appreciated mentors' efforts.

(d) Based on the students' evaluation and comments, we believe we have a very successful training program. The success of the training program is clearly shown that our **current** and **former** NPCRP-CAU trainees were eagerly participating to various scientific meetings and

gave posters (**Key Research Accomplishments, Reportable Outcomes and Appendices #8-14**). We are very pleased with the success of our Training Program, a joint effort by our colleagues and stuff member.

4. **KEY RESEARCH ACCOMPLISHMENTS:**
(**For 2014 Annual Report**)

- All CAU students attended the Monday seminar offered by the INBRE program through the entire period.
- All CAU students visited different research labs and biopharmaceutic companies offered by the INBRE program through the entire period.
- All CAU students attended Tuesday noon seminar offered by the UNMC Summer Undergraduate Research Program through the entire period.
- These students also visited regional universities and local BioPharm companies to expand their knowledge and scopes in future career developments.
- The UNMC INBRE IN ROADS, an INBRE communication, introduced the CAU students and reported the NPCRP efforts and the interactions between two programs.
- All CAU students prepared their results in posters.
- Our current CAU trainee Ms. Marisha Morris gave a poster presentation in the The Annual Biomedical Research Conference for Minority Students Annual National Symposium, November 15, 2013.
- Our current and former CAU trainees gave poster presentations in the 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014.
-

5. **CONCLUSION:**

The purpose of this award is to train HBCU undergraduate students from CAU to gain hand-on experience in performing PCa research in a research-intensive focus group, the UNMC Nebraska Prostate Cancer Research Program (NPCRP). We are very pleased with the outcomes for the continuing success of the training program at UNMC. These students have received training not only in the lab but also in the class room in the format of seminars and visiting biotechnology companies. Our goals of training are to encourage and to prepare HBCU undergraduate students for academic career in graduate school or medical school with training in, and understand of, prostate cancer research. We propose that by this way, we can increase the number of PCa researcher from the minority group at both the basic science and the clinical science levels. With this award support from the DOD PCa Research Program, as evidenced by the scientific outcomes of student posters and student comments, we are very excited by the success of our training program. It is clearly evidenced by current and former students who are eagerly participating various scientific meetings and gave posters as described in accomplishments in sections: **Key Research Accomplishments, Publication, Abstracts and Presentations, and Reportable Outcomes (Appendices #8-14**). The results of research together have been included in a solid, peer-reviewed scientific article (**Appendix #15**). We are expecting that more exciting results will be done in the up-coming years.

6. **PULICATIONS, ABSTRACTS, AND PRESENTATIONS:**
(All NPCRP Trainees including current and former are identified in **bold**.)

PULICATION:

a. Muniyan, S., Chou, Y.W., Ingersoll, M.A., **Devine, A.**, **Morris, M.**, Odero-Marah, V.A., Khan, S.A., Chaney, W.G., Bu, X.R., Lin, M.F. (2014). Antiproliferative activity of novel imidazopyridine derivatives on castration-resistant human prostate cancer cells. Cancer Letters 353: 59-67. (PMID: 25050738) (NIH MS615152, Publ.ID: CAN11924)

ABSTRACTS AND PRESENTATIONS:

a. Vo, B.T., Boseman, M., **Leath, C.**, Battle, S., Khan, S.A. (2014). Intracellular mechanisms of arcadia abolished TGF-beta induced proteasome degradation of Ski protein in prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.62.
b. Chunduri, H., Vo, B.T., **Leath, C.**, Khan, S.A. (2014) Gai is critical for TGF-beta1, PGE2, OXT and EGF induced migration in prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.66.
c. Ghotbaddini, M., Tran, C., Richmond, O., **Aaron, LaTayia**, Powell, J.B. (2014). Inhibition of constitutive aryl hydrocarbon receptor (AHR) signaling attenuates androgen independent signaling and growth in C4-2 prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, mp.70.
d. **Leath, C.**, Jiang, C., Davis, J. Khan, S. (2014). Inhibiting RAC 1-GTPase activity in ovarian cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.83.
e. **Loyd, Q.**, Smith, B.N., Henderson, V., McKeithen, D., **Morris, M.**, Nagappan, P., and Odero-Marah, V.A., (2014) Snail transcription factor regulates cathepsin L and maspin in prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.84.
f. **Morris, M.**, Muniyan, S., Dwyer, J.G., Bu, X., Lin, M.F. (2014). Novel imidazopyridine derivatives inhibit androgen-independent prostate cancer cell proliferation. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p. 87.
g. **Wells, J.**, Petrosyan, A., Cheng, P.W. (2014) Non-muscle myosin IIA-mediated golgi fragmentation in advanced prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.98.

PRESENTATION:

a. **Marisha Morris**, Sakthivel Muniyan, Jennifer G. Dwyer, Xiu Bu, Ming-Fong Lin (2013). Novel imidazopyridine derivatives inhibit androgen-independent PCa cell proliferation. The Annual Biomedical Research Conference for Minority Students Annual National Symposium, November 15, 2013.

7. **INVENTIONS, PATENTS AND LICENSES:**
None.

8. **REPORTABLE OUTCOMES:**
(**For 2014 Annual Report**)

- All our CAU trainees had gained valuable hand-on experience in PCa research.
- They learned translational research in PCa, including biomarker discovery, molecular pathogenesis, signaling transduction and experimental therapy.
- CAU student Ms. Marisha Morris gave poster presentations in the The Annual Biomedical Research Conference for Minority Students Annual National Symposium, November 15, 2013.
- With inputs of CAU trainees, our research results have identified a novel group of compounds that may be used in advanced PCa therapy.
- Our results have been published as a peer-reviewed article for potential PCa therapy. (Publication #1: Muniyan, S., et.al. (2014). Antiproliferative activity of novel imidazopyridine derivatives on castration-resistant human prostate cancer cells. Cancer Letters 353: 59-67.)
- Our current and former CAU trainees gave poster presentations in the 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014.
- In the 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, a total of 7 posters were contributed by our CAU trainees.
-

9. **OTHER ACHIEVEMENTS:**
None. There is no other achievement.

10. **REFERENCES:**

1. Dillard, P.R., **Lin, M.F.**, and **Khan, S.A.** (2008). Androgen-independent prostate cancer cells acquire the complete steroidogenic potential of synthesizing testosterone from cholesterol. Mol. Cellu. Endo. 295:115-120.
2. Siegel, R., Naishadham, D., and Jemal, A. (2012). Cancer Statistics, 2012. CA Cancer J Clin 62: 10-29.

11. **APPENDICES:**
(**For 2014 Annual Report**)

1. Appendix #1: Announcement by BMB for Dr. Shafiq Khan's scientific presentations at UNMC.
2. Appendix #2: Itinerary for Dr. Shafiq Khan meeting with NPCRP faculty members for discussing future collaborations.
3. Appendix #3: Poster prepared by CAU trainee Chelesie Leath
4. Appendix #4: Poster prepared by CAU trainee Jaclyn Welles
5. Appendix #5: Poster prepared by CAU trainee Tashika Robinson
6. Appendix #6: Poster prepared by CAU trainee Mohammed Ghislain Djibo.
7. Appendix #7: CAU student evaluations.

8. Appendix #8 (p.62): Poster presentations by Ms. Chelesie Leath in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
9. Appendix #9 (p.66): Poster presentations by Ms. Chelesie Leath in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
10. Appendix #10 (p.70): Poster presentations by Ms. LaTayia Aaron, our 2011 trainee, in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
11. Appendix #11 (p.83): Poster presentations by Ms. Chelesie Leath in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
12. Appendix #12 (p.84): Poster presentations by Quentin Loyd in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
13. Appendix #13 (p.87): Poster presentations by Ms. Marisha Morris, our trainee, in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
14. Appendix #14 (p.98): Poster presentations by Ms. Jaclyn Wellws in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
15. Appendix #15: Peer-reviewed scientific publication with inputs from CAU trainees (Cancer Letters, 2014).

12. TRAINING AWARD:

List of Training Activities include seminar and visiting.

1. Appendix #16: An example of INBRE schedule including seminar and visiting.
2. Appendix #17: An example of Summer Undergraduate Student Program including seminar and visiting.
3. Appendix #18: A list of NPCRP mentors and their research expertise.

Lin, Ming-Fong

From: Hankins, Karen L
Sent: Monday, July 08, 2013 10:38 AM
To: Batra, Surinder K; Caplan, Steven H; Chaney, William G; Cheng, Pi-Wan; Christman, Judith K; Cox, G Stanley; Datta, Kaustubh; Jain, Maneesh; Klinkebiel, David L; Lin, Ming-Fong; MacDonald, Richard G; Mehta, Parmender P; Mott, Justin L; Naslavsky, Naava; Palanimuthu Ponnusamy, Moorthy; Ramaley, Robert F; Sorgen, Paul L; Steinke, Laurey A; Teoh-Fitzgerald, Melissa M; Agarwal, Ekta; Al-Mugotir, Mona H; Bahl, Kriti; Cai, Bishuang; Chugh, Seema; Cruz, Eric; Das, Srustidhar; Gupta, Suprit; Haridas, Dhanya; Heimann, Nicholas B; Huang, Huocong; Ji, Weike; Joshi, Suhasini; Katoch, Parul; Krishn, Shiv Ram; Li, Hanjun; Lu, Sizhao; Mohapatra, Bhopal C; Pai, Priya; Panapakkam Giri Dhar, Sai Srinivas; Rajbhandari, Nirakar; Ray, Anuttoma; Reinecke, James B; Remmers, Neeley A; Roy, Sohini; Saxena, Sugandha; Souchek, Joshua J; Struble, Lucas R; Suresh, Anand; Talbott, Heather A; Tom, Eric C; Vaz, Arokiapriyanka; Wehrkamp, Cody J; Wiest, Edwin J; Xie, Shuwei; Zavorka, Megan E; Zhang, Yinbo; Dodson, Amy L; Fontaine, Michele; Gardner, Jeanette L; Jones, Atiim D; Hankins, Karen L; Katafiasz, Dawn M; Kelsey, Linda S; Klima, Susan; Mallya, Kavita; Polavaram, Navatha Shree; Smith, Mary A; Talaska, James R; Taylor, Janice; Zach, Sydney J
Subject: BMB Seminar TODAY

Importance: High

Please join the Biochemistry and Molecular Biology department as they welcome **Dr. Shafiq A. Khan.**

Dr. Khan is a Professor in the Department of Biological Sciences at Clark Atlanta University. Presenting a seminar entitled **"Signaling Transduction in Prostate Cancer and the Effort of Biodepository of Minority Specimens."**

July 8, 2013
DRC… Room 1004
12:00 pm - 1:00 pm

Karen L. Hankins
Office Associate I
Department of Biochemistry & Molecular Biology
University of Nebraska Medical Center
985870 Nebraska Medical Center
Omaha, NE 68198-**5870**
P: 402-559-4417
F: 402-559-6650
E-mail:karen.hankins@unmc.edu
****

Itinerary for Dr. Shafiq Khan
Clark Atlanta University

Sunday, July 7, 2013

3:10 p.m.	Arrive on Southwest Flight 3500 (Departure at 12:15pm). Dr. Ming-Fong Lin will pick you up from the airport and drive you to the hotel.
Hotel Reservations:	Courtyard Omaha Downtown 101 South 10th Street Omaha, NE 68102 402-346-2200 Confirmation #89697004
	Dinner with Drs. Chaney, Davis and Khan

Monday, July 8, 2013

8:30 a.m.	Dr. Lin will pick you up and drive you to UNMC for breakfast with faculty mentors.
9:00 a.m.	Continental breakfast with faculty mentors – DRC 7003 Faculty mentors: Drs. Surinder Batra, John Davis, Pi-wan Cheng, Kaustubh Datta, William Chaney, Ming-Fong Lin and Yaping Tu (Creighton University)
10:00 a.m.	Dr. Yaping Tu, Creighton University – DRC 7003
10:45 a.m.	Dr. Batra, 7005 DRC1
11:45 a.m.	Prepare for seminar presentation
12:00 p.m.	Seminar presentations – DRC 1004
1:15 p.m.	Meeting with CAU students – DRC1 7003
2:30 p.m.	Meeting with Dr. Davis – DRC1 7003
3:15 p.m.	Meeting with Dr. Datta – DRC1 7003
4:00 p.m.	Meeting with Dr. Cheng – DRC1 7003
4:45 p.m.	Dr. Lin at DRC I 7003
5:30 p.m.	Return to hotel or go with Dr. Davis.
6:00 p.m.	Dinner with Drs. Khan, Turpen and Lin.

Tuesday, July 9, 2013

9:45 a.m.	Depart on Southwest Flight #539.

Inhibiting RAC 1- GTPase Activity in Ovarian Cancer Cells

Chelesie Leath, Chao Jiang, Dr. John Davis ,Obstetrics and Gynecology Research Department
University of Nebraska Medical Center, Omaha, Nebraska 68198-5800

ABSTRACT

Rho proteins have been described as "molecular switches" and play a role in many common cellular functions such as cell proliferation and survival ,apoptosis and gene expression , but also in a number of diseases, neurological disorders and different types of cancer. Previous studies have shown that Rho GTPases are over expressed in breast and colorectal cancers. The mechanisms involved in regulating Rho GTPases activity are still largely unknown. Because of their multifunctional properties, these proteins are thought to be ideal candidate for anti-cancer therapeutics and drug development. To date, most efforts focused on inhibitors that block indirectly, by targeting enzymes involved in post-translational processing or downstream protein kinase effectors. Identifying small molecular inhibitors to directly target RHO GTPase functions may be ideal for inhibiting progression of aggressive cancer into more advanced stages. Our preliminary studies show that RAC 1 and its splice variant RAC1 b are highly expressed in ovarian cancer cells. The purpose of this study is to determine the effective of small molecular inhibitors NSC23766 and EHT 1864 in human ovarian surface epithelial and ovarian cancer cells, in the presence and absence of anti-cancer drugs, as a potential therapy approach.

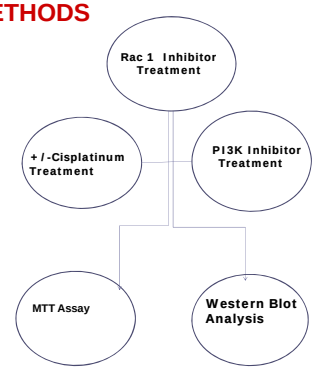
INTRODUCTION

The Rho family of GTPases is a family of small (~21 kDa) signaling G protein that belongs to the RAS superfamily. Members of the Rho GTPase family can be activated by Growth factors, Cytokines, Adhesion molecules, Hormones, Integrins, G-proteins and other biologically active substances and regulate a wide range of biological processes, including Reorganization of the Actin Cytoskeleton, Transcriptional Regulation, Vesicle Trafficking, Morphogenesis, phagocytosis, and production of free radicals and oxygen species. GTPases exist in two forms: active and inactive. GTPases are inactive in a GDP –bound state until activated by GEFs , guanine exchange factors , thus becoming GTP-bound. Activated GTPases can interact through upstream intracellular signaling and interactions with downstream effectors of multiple pathways, to regulate a specific function.

One of the best categorized members of the Rho family is RAC 1. RAC 1 drives actin polymerization and formation of lamellipodia, promotes cell-cell adhesion, breakdown and migration of different carcinoma cells. RAC 1 is activated by Rac specific GEFs Tiam1 or Trio. RAC1 GTPase mediates key cellular processes in response to upstream regulators such as Growth Factors, Integrins and HA (Hyaluronic Acid)-binding receptor CD44 and is a key downstream target/effectors of PI3K/Akt/mTOR pathways. RAC1b is a tumor associated, constitutively active and transforming splice variant of Rac-1 that promotes cellular transformation. It includes a 19 amino acid insert (57 base pairs) behind its switch 2 domain, an important for interactions with effectors and regulators.

MATERIALS AND METHODS

Cell Line	Cell Type
IGROV-1	originating from an ovarian carcinoma of a 47-year-old Exhibits highly tumorigenic properties.
SKOV3	Human ovary cancer cell line SKOV-3 derived from the ascitic fluid of a 64 year old Caucasian female ;Epithelial-like morphology, is also able to grow in soft agar, an indicator of transformation and SK-OV-3 cells are resistant to tumor necrosis factor and to several cytotoxic drugs including diphtheria toxin, cis-platinum and adriamycin. ;Tumorigenic
TOV21G	Human ovary tissue from a 62-yr old female; stage III,primary malignant adenocarcinoma ;clear cell carcinoma
Hose T80	Immortalized Human ovarian surface epithelial cell
Hose 969	Primary Human ovarian surface epithelial cell



RESULTS

The Effects of the RAC1 Inhibitor NSC23766 on Viability in Ovarian Epithelial Cell Lines

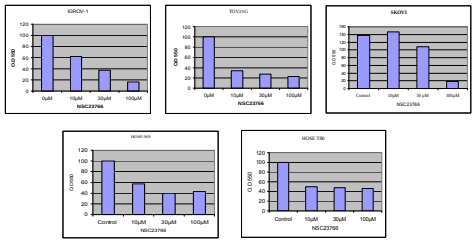


Figure 1. Graphical data from MTT assay performed to determine the effects of RAC 1 Inhibitor NSC23766 on human ovarian surface epithelial and ovarian cancer cells. NSC23766 decreased viability in ovarian cancer cells, IGROV-1, SKOV3, TOV21G, and also in human ovarian epithelial cells.

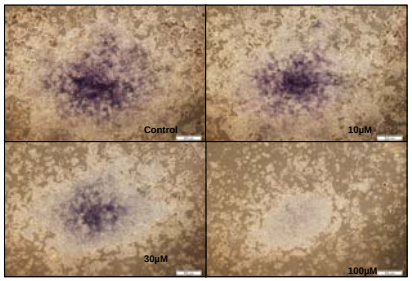


Figure2. MTT assay results after treatment of NSC23766 in IGROV-1 cells. Cells were treated with 0, 10, 30 and 100 μM of nsc23766 for 48 hours.

The Effects of Treatment with NSC23766 in Combination with Anticancer drug Cisplatinum on Viability in Ovarian Epithelial Cancer Cell Lines

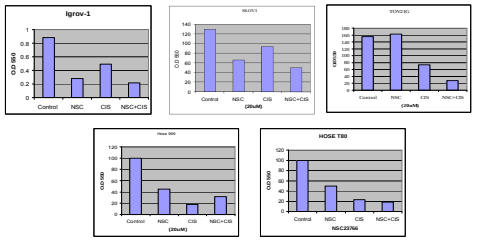


Figure 3. Graphical data from MTT assay performed to determine the effects of RAC1 Inhibitor NSC237766 against cytotoxic drugs human ovarian surface epithelial and ovarian cancer cells. NSC23766 , in combination with Cisplatinum , was more effective in decreasing viability.

The Effects of NSC23766 On Cell Signaling in Ovarian Cancer Cells

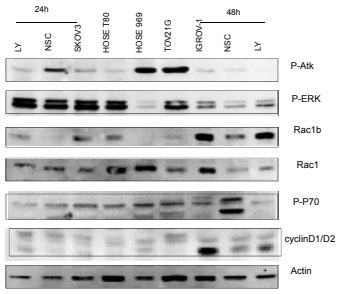


FIGURE 4 . Cells were treated with NSC23766 (50uMl) for 24 or 48 hours. B- actin acted as the internal control. Western Blot analyses revealed reduction of activation of p-AKT , p-ERK, RAC1, and RAC 1B in IGROV-1 cells and RAC 1 and RAC1B in SKOV3.

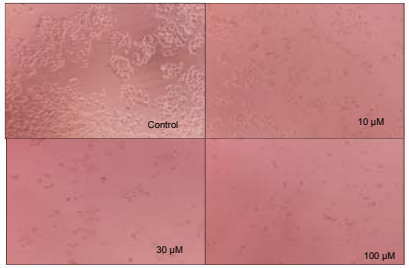


Figure 5. Results from MTT assay shows morphological difference upon treatment with RAC1 Inhibitor NSC23766.

The Effects of Treatment with EHT 1864 on Viability in Ovarian Epithelial Cell Lines

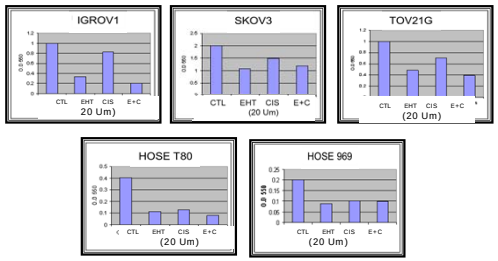


Figure 6 Graphical data from MTT assay performed to determine the effects of RAC1 Inhibitor EHT 1864 on human ovarian surface epithelial and ovarian cancer cells. RAC1 NSC23766 decreased viability in ovarian cancer cells, IGROV-1, SKOV3, TOV21G, and also in human ovarian epithelial cells

The Effects of EHT 1864 On Cell Signaling in Ovarian Cancer Cells

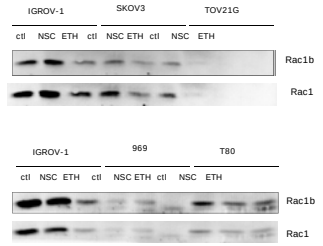


FIGURE 7 . Cells were treated with NSC23766 (50uM)and EHT 1864(50uM) for 48 hours. Western Blot analyses revealed reduction of total RAC 1 and RAC1B in human ovarian surface epithelial and ovarian cancer cells.

The Effects of Treatment with EHT 1864 in Combination with Anticancer drug Cisplatinum on Viability in Ovarian Epithelial Cancer Cell Lines

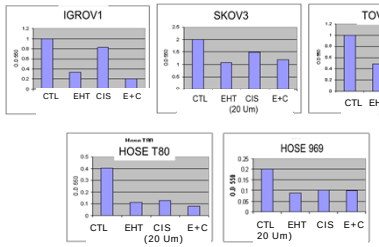


Figure 8. Graphical data from MTT assay performed to determine the effects of RAC 1 Inhibitor EHT 1864 on human ovarian surface epithelial and ovarian cancer cells. RAC1 inhibitor EHT 1864 decreased viability in ovarian cancer cells, IGROV-1, SKOV3, TOV21G, and also in human ovarian epithelial cells .

SUMMARY/CONCLUSIONS

- Treatment with NSC23766 and EHT 1864 reduced viability in both normal human ovarian surface epithelial and ovarian cancer cells.
- In some cases, EHT 1864 and NSC23766 Increased sensitivity of ovarian cancer cells to anti-cancer therapeutic drug.
- Combination treatment of Rac Inhibitors and Anti-Cancer Drug ,Cisplatinum, was more effective in decreasing proliferation than treatment of Rac Inhibitors alone.

ACKNOWLEDGMENTS

I would like to thank Dr. John Davis , Chao Jiang , the entire Davis and Wang lab, Dr. Ming Fong Lin , Dr. William Chaney ,and the Dept. of Biochemistry at University of Nebraska Medical Center. Special Thanks to Dr. Shafiq Khan , faculty and staff at Center for Cancer Research and Therapeutic Development at Clark Atlanta University, in Atlanta, Georgia. This study was supported by the Department of Defense grant # PC121645.

Non-muscle myosin IIA-mediated Golgi fragmentation in advanced prostate cancer cells

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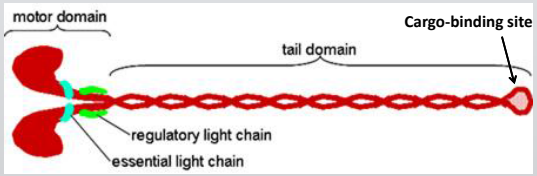
[1]Clark Atlanta University, Atlanta, GA; [2]VA Nebraska Western Iowa Health Care System, Omaha, NE; and [3]Department of Biochemistry and Molecular Biology, College of Medicine, Omaha, NE

INTRODUCTION

Prostate cancer is the second leading cause of cancer death among men older than 55 in the United States. Androgen ablation is the main therapy for prostate cancer[1]. However, androgen refractory will develop several years later. Once it occurs, the cancer is incurable. Therefore, there is a pressing need to understand the biology of the hormone refractory prostate cancer to aid the development of therapy for these patients. The three the most commonly used cell lines for studying human prostate cancer are LNCaP, PC3, and Du145[6]. They are derived from prostate tumors metastasized to lymph node, brain and bone, respectively. The LNCaP cells, which are sensitive to androgens, are less tumorigenic than DU145 and PC3 cells, which are androgen refractory[6]. Golgi disorganization has been reported in many cancers. But the mechanism was not known. In a recent study, we showed fragmented Golgi in both colon tumors as well as metastatic carcimona HT-29 cells[11]. The fragmented Golgi was reversed to compact morphology after treatment with a non-muscle myosin IIA inhibitor Blebbistatin (BB)[11]. This observation prompted us to ask the question of whether same phenomenon occurs in prostate cancer cells.

As shown next, non-muscle myosin IIA is a motor protein consisted of two heavy chains and two pairs of light chains [6]. The N-terminal region is the motor domain involved in walking on actins cable and the C-terminal region is the cargo-binding domain. Non-muscle myosin IIA is involved in Golgi remodeling by interacting with the cytoplasmic tails of Golgi resident proteins under basal conditions[5] and Golgi fragmentation under stress.



HYPOTHESIS

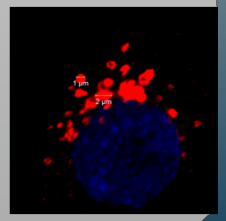
The goal of this study was to test the hypothesis that the Golgi in prostate cancer cells is fragmented and the fragmented Golgi can be reversed to compact morphology by inhibition of non-muscle myosin IIA.

METHODS

Culture and BB treatment of LNCaP, PC3 and DU145 cells:

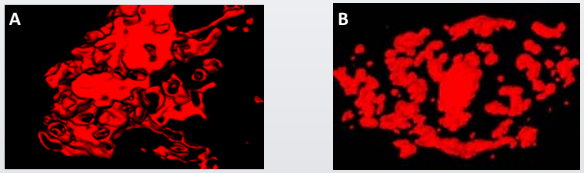
Confocal immunofluorescence microscopy analysis of the Golgi morphology:

Determination of Golgi fragmentation: AS shown in the Figure, the Golgi fragments with sizes between 1-2 µm were counted.



RESULTS

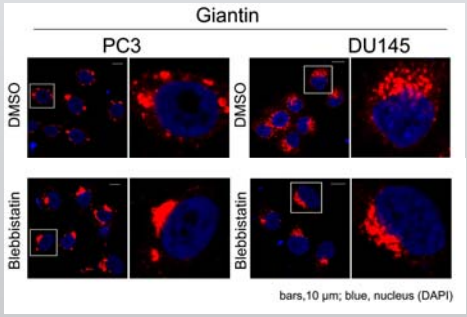
3-D reconstruction of the Golgi morphology in (A) LNCaP and (B) PC3 & DU145 cells



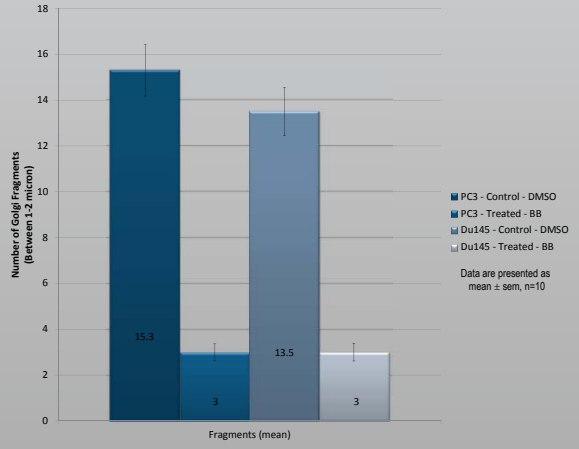
Compact Golgi in LNCaP cells

Fragmented Golgi in PC3 and Du145 cells

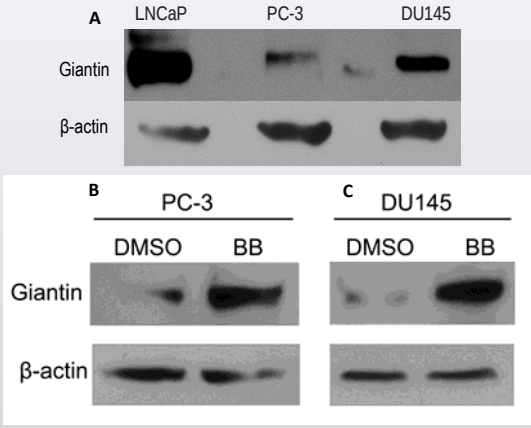
Blebbistatin treatment of PC3 and DU145 cells restores the compact Golgi morphology



Quantification of Golgi fragments in PC3 and DU145 cells treated with DMSO or Blebbistatin



Western blot analysis of Giantin in LNCaP, PC3 and DU145 (A), and PC3 (B) and DU145 (C) cells after treatment with DMSO or BB



SUMMARY

- LNCap cells have compact Golgi while PC3 and DU145 cells exhibit fragmented Golgi, which can be restored by inhibition of NMIIA.
- Basal level of Giantin is higher in LNCaP than those in PC3 and DU145 cells. Inhibition of NMIIA increases Giantin levels in these two cells.

CONCLUSION

Advanced prostate cancer cells exhibit NMIIA-mediated Golgi fragmentation .

FUTURE STUDY

- To determine if fragmented Golgi is associated with altered glycosylation.
- To determine if raising Giantin level can restore the compact Golgi morphology in advanced prostate cancer cells.

ACKNOWLEDGMENTS

The work was supported by DOD PC121645 "The Nebraska Prostate Cancer Research Program" and VA Merit Award 1I1BX000985 and the State of Nebraska LB506 grant.

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NRP-2 Stabilizes CXCR4 and Promotes Prolonged Signaling and Increased Migration

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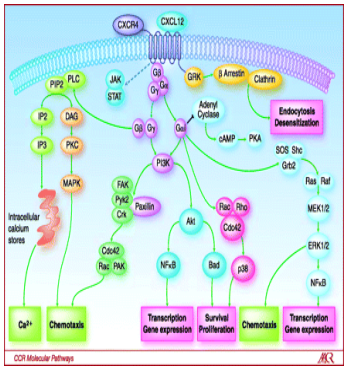
[1] Clark Atlanta University, Atlanta, GA; [2] University of Nebraska Medical Center, Omaha, NE

Background

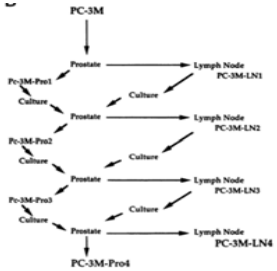
C-X-C chemokine receptor type 4 (CXCR-4) is a molecule expressed on the cell surface where specific factors (soluble) can bind and interact with it. CXCL12 is a ligand for CXCR4. CXC4 is the only receptor known for CXL12 (aka) SDF1. NRP-2 stabilizes CXCR4 on the surface of the cell and promotes prolonged signaling, and increased migration. We questioned how CXCR4 is regulated in the presence of NRP-2 interacted with it at the cell surface level. Based on the hypothesis of them interacting, we knocked down NRP-2 to see how the activity of CXCR4 down regulated.

CXCR4



Methods

How PC3 M LN4 cells were made

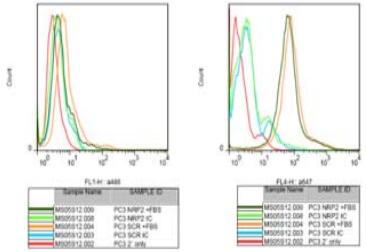


Pettaway, et al. Clinical Cancer Research 1996

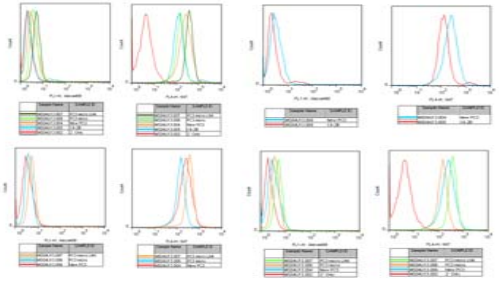
We want to identify different cell lines because we observed that there is no significant difference between NRP-2 depleted cells and controls. We decided to look for cells that express more NRP-2 on their surface.

Results

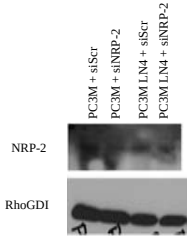
PC3 FACS Assay



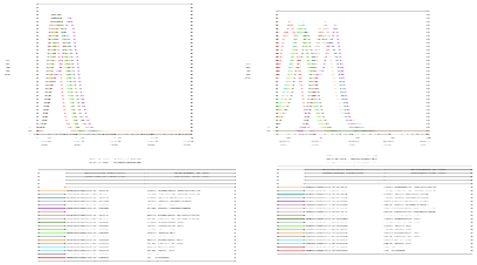
Lamp-2 functions in autophagy



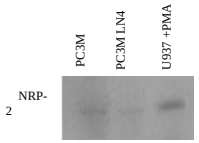
Western Blot Examining KD efficiency



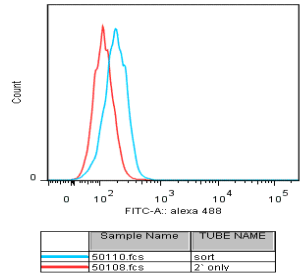
LNCaP C4-2B FACS Results Starved Cells



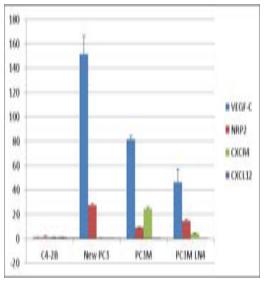
Western Blots Examining NRP-2 levels



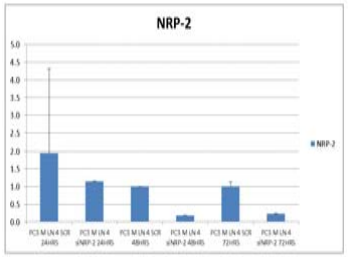
Sorting of PC3M LN4 Cells based on surface expression of NRP-2



mRNA Expression Panel



Depletion of NRP-2 in PC3M LN4



Summary

By applying the research methods aforementioned to support our hypothesis, we were able to determine that NRP-2 stabilizes CXCR4 on the surface of the cell and promotes prolonged signaling, and increased migration. In conducting further research, we hope to transfect sorted cells with NRP-2 siRNA for 48 hours prior to FACS analysis. Following that, we will examine the effect of NRP-2 depletion on CXCR4 levels. We will also use sorted cells in migration assay to determine whether NRP-2 expression has an effect on migration along SDF-1 gradients.

Acknowledgement

This research was funded by DOD grant award #PC 121645.

Effects of Zoledronic Acid (Zometa) in Enhancing the Efficacy of Radiotherapy and Chemotherapy in Prostate Cancer

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Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska, U.S.A

Introduction

- Prostate cancer (PCa) is the second highest leading cause of cancer-related deaths among U.S. males and there is an estimated of 238,590 new cases and 29,720 deaths for the year 2013 (1).
- Treatments available to prostate cancer patients: watchful wait or active surveillance, surgery, radiotherapy, chemotherapy, biologic therapy and androgen deprivation therapy (ADT).
- Despite the multiple means of therapy, most PCa cases eventually recur after treatment or develops a resistance against available therapies. Such complications being in effect, we investigated on ways to decrease the resistance of PCa to therapy and reduced the ability of biochemical tumor recurrence.
- Recent studies has demonstrated that farnesyl diphosphate synthase (FDPS), the key enzyme of the mevalonate pathway, contribute for PCa progression and biochemical recurrence (2,3).
- Drugs used to treat diseases related to bone resorption, such as zoledronic acid, more commonly known as zometa, a nitrogen containing bisphosphonate, inhibit FDPS through Inhibition of the mevalonate pathway and prevention of post-translational prenylation of GTP-binding Proteins (4).
- The downstream protein of FDPS such as PAK has shown to be involved in tumor growth and micro-invasion, by enhancing the activity of MMP-9 in PCa (5).
- Furthermore, identification of a novel sterol C4-methyl oxidase (SC4MOL) has been predicted to be a binding partner and down stream of FDPS in cholesterol biosynthesis pathway(6).
- Moreover, zometa has been tested with several drugs in different cancers to enhance tumor growth inhibition. Therefore, we investigated its effects with CI-1033, a pan tyrosine kinase inhibitor, which irreversibly inhibits all the four members of Epithelial Growth Factor Receptor (EGFR) family (7).
- To identify the synergistic effect of zometa with radiation/chemothepy, we further investigated the ERK and AKT signaling mechanism on prostate cancer cells treated with zometa and CI 1033 (alone and in combination)(7).

Hypothesis: Tumor recurrence in prostate cancer develops after radio and chemotherapy. Hence, there is an urgent need to develop means to sensitize cancer cells to the available therapies, in order to decrease the odds of biochemical recurrence. *Based on the previous studies of the effect of nitrogen- containing Bisphosphonates on FDPS we hypothesized that zometa could inhibit the overexpression of FDPS and its downstream proteins Pak and its interacting partner SC4MOL in radiation therapy and when used along with the chemotherapeutic agent CI-1033 it could further abrogate both proliferative and survival signaling in prostate cancer.*

Methods

- Clonogenic survival assay of 1,3,5,7 Gy on various prostate cancer cell lines PC3, 22RV1, E4 and CE2 (Mouse syngeneic cells), DU145 and C42B was performed to determine the levels of radiation necessary to generate resistant cell lines.
- A radiation sub line of 22RV1RR and CE2RR (RR-Radiation Resistant) were generated with 3Gy/Day for five days consecutively.
- MTT assay was performed to determine IC_{20} and IC_{50} values of zometa and CI-1033 respectively.
- Western blot analysis was performed to investigate the effect of zometa and CI1033 on its downstream signaling targets.
- MTT proliferation assay was performed at 48 and 72h on the 22RV1RR and CE2RR cells with zometa and CI1033 (alone and in combination) to observe percentage of inhibition.
- Colony formation assay was performed on prostate cancer cells treated with CI-1033 and zometa using standard protocol.

Results

1.

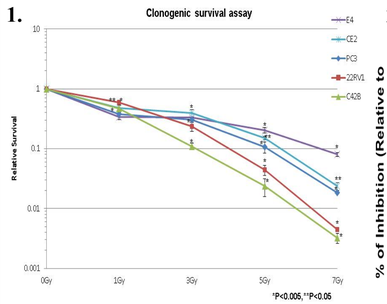
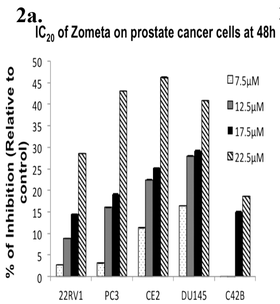


Figure 1:. Cells were exposed with the indicated doses of radiation. After 10 days of incubation in complete medium cells were stained with 0.4% crystal violet and the colonies containing >50 cells were counted. AR positive 22RV1 and CE2 cells at 3Gy were considered for further radiation studies.

2a.



2b.

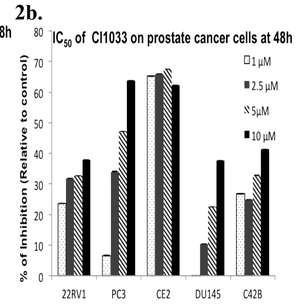


Figure 2: (a) Determination of IC20 value of zometa through MTT assay at 48h show an IC_{20} value of 17.5 μM for 22RV1 and 12.5 μM for CE2 cells. **(b)** MTT assay demonstrated an IC_{50} value of 5 μM for 22RV1 and CE2 cells at 48h.

3.

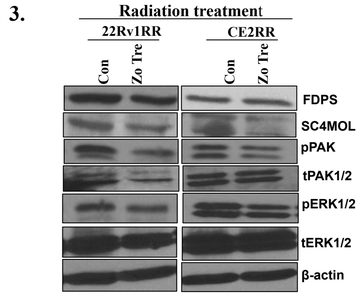


Figure 3: Western blot analysis of Zometa inhibition on targeted proteins. We observed a decrease in the expression of FDPS, SC4MOL, pPAK, pERK compared to the control in both 22RV1 and CE2 cells. No change in the level of expression in total ERK in both 22RV1 and CE2 cells , in contrast a significant decrease of total PAK was also observed in 22RV1 alone.

4.

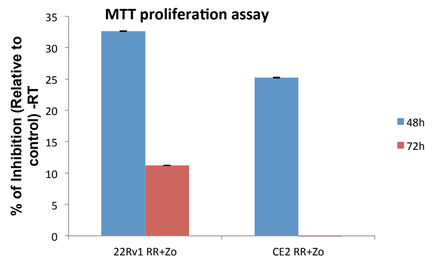
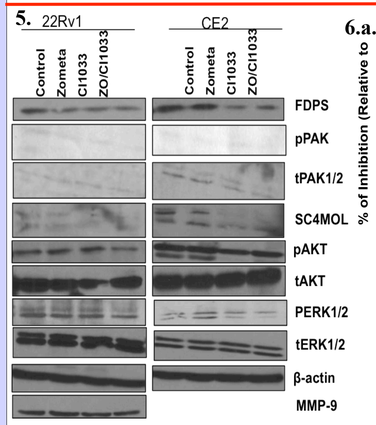
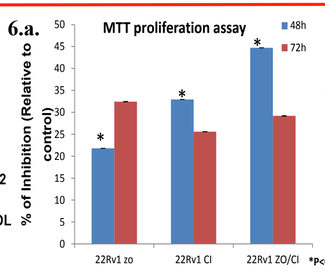


Figure 4: MTT proliferation assay at 48h and 72h: The percentage of inhibition was increased in the resistant sub lines relative to the control when zometa was used at 17.5 μM for 22RV1 and 12.5 μM for CE2 on a radiation resistant cells.

5.



6.a.



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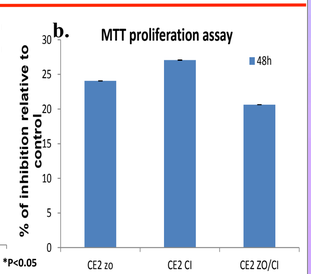
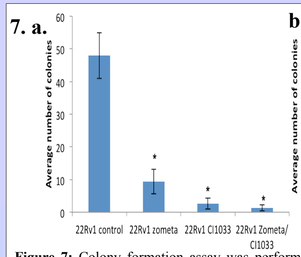


Figure 6: (a&b) Effect of CI-1033 (5 μM) and zometa (17.5 μM for 22RV1 and 12.5 μM for CE2) on the proliferation of prostate cancer cell lines *in vitro*. The cells were treated either alone or in combination with the indicated concentrations of zometa and CI1033 for 48h and 72h, and the proliferation of cells were determined by the MTT assay. The percentage of inhibition for 22RV1 when treated with zometa and CI1033 is statistically significant at 48 h in comparison to their use as a single agents. In contrast, we could not able to demonstrate similar proliferative activity in CE2 mouse syngeneic cell lines at 48h, no decrease in cell proliferation was observed at 72h.

Figure 5: In vitro effects of zometa in combination with pan EGFR inhibitor CI1033 on cholesterol biosynthesis and EGFR pathway in the 22RV1 and CE2 prostate cancer cells : Western blot analysis revealed that zometa combined with CI-1033 had greater effects in down regulating FDPS, SC4MOL and decrease the phosphorylation of PAK in both 22RV1 and CE2 compared to the use of single agent. The combination treatment with CI1033 with zometa further significantly down regulates the phosphorylation levels at serine 473 of AKT and pERK1/2. However, there is no change in the total AKT and ERK levels in control and treatment conditions. Additionally, we observed a significant decrease in the expression of in MMP-9, which signifies the decrease in the migratory property in 22RV1cells.

7. a.



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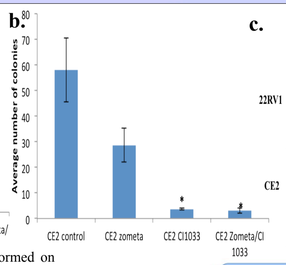

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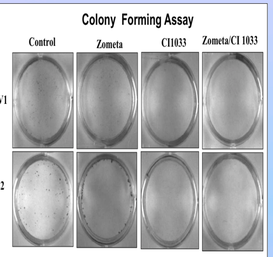
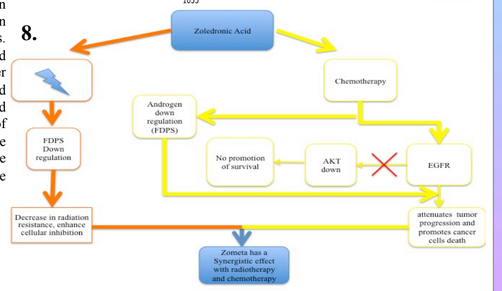


Figure 7: Colony formation assay was performed on 22RV1 and CE2 cells with 500 cells seeded per plate in a 12-well plate and grown for a period of 10 days. Colonies were visualized by crystal violet staining and the assay revealed a significant decrease in the number of colonies in the zometa and CI-1033 group compared to the control, zometa treated, or CI-1033 treated groups. **(a&b)** Graph of the average number of colonies formed under various treatments versus the control in 22RV1 and CE2 cells; **(c)** Representative images of the plates under various treatments versus the control in 22RV1 and CE2 cells.

Figure 8: Schematic representation depicting the mechanism of inhibition of prostate cancer cells by zometa in synergy with radio and chemotherapy by down regulating FDPS and its downstream signaling targets.

8.



Summary and Conclusion

- Radiation resistant sub-lines were developed from the clonogenic survival assay at 3 Gy radiation.
- Zoledronic Acid showed to be an effective drug at both 48h and 72h but the optimal effect on proliferation inhibition was observed in 48h of PCa cells.
- Our western blot results demonstrates that zometa affects the expression of the tumor promoter FDPS and its downstream targets (PAK and SC4MOL) by inhibiting protein prenylation in both radiated and chemotherapy treated cells.
- Notably, zometa combine synergistically to impede cellular proliferation of radiation resistant cells at 48h and 72h. The decreases in proliferation of radiation resistant cells were also associated with down regulation of the proliferative (ERK) and survival (AKT) signals.
- 22RV1 cells treated with CI-1033 and zometa showed a significant higher inhibition of cellular proliferation and decreased colony forming ability in CI1033 and zometa alone compared to untreated cells. Hence, a synergistic inhibition of the cell proliferation and the colony-forming potential of prostate cancer cells could be enhanced if zometa is treated along with a pan EGFR family inhibitor. In contrast, CE2 cells have demonstrated with lower inhibition of cellular proliferation.
- Zometa achieves its better synergism with radiotherapy rather than chemotherapeutic agent as implied by the results of Figure 3 and 4.

The results obtained demonstrates that zometa has the potential to decrease prostate cancer cells resistance to radiation and chemotherapy, and decreasing the odds of biochemical tumor recurrence by the inhibition of the tumor growth and proliferation promoter FDPS downstream signaling. To be more precise these results indicates that zometa effectively inhibit the growth of PCa cells in vitro by targeting at molecular level at lower concentration (FDPS) and synergizing with radiation to provide enhanced antitumor efficacy in locally advanced prostate cancer patients.

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Acknowledgement

Nebraska Prostate Cancer Research Program supported by grant PC121645

2013 Nebraska Prostate Cancer Research Scholars Program

Student Evaluation

1. **How satisfied are you with the Nebraska Prostate Cancer Research Scholars Program(NPCRSP)?**

Very Satisfied **Satisfied** **Dissatisfied** **Very Dissatisfied**

2. **How did you originally learn about the NPCRSP?**

Through the cancer research center.

3. **Did you have a clear set of expectations of the NPCRSP when you became a NPCR Scholar?**

yes, I did. I wanted to leave the program with a better understanding of research than I had before.

4. **How would you rate your research experience as it relates to helping you make decisions related to your education and career plans?**

I think it was a very important research experience towards my future goals.

5. **What do you consider the main benefit(s) of the NPCRSP?**

1. You get hands on research experience.
2. You get to experience a new location and culture outside of your comfort zone
3. You get to speak to professionals in the field and get advice about future career decisions.
4. You get thorough knowledge on an aspect of science that you may not have known much about before.

6. **What suggestions would you give for the program for next summer?**

* I would suggest that only that perhaps they would be a little more lenient about which seminars we are required to go to. Not all of the seminars are for everyone.
* This program was an amazing experience and I'll be forever greatful. Thank you

2013 Nebraska Prostate Cancer Research Scholars Program

Student Evaluation

1. **How satisfied are you with the Nebraska Prostate Cancer Research Scholars Program(NPCRSP)?**

   Very Satisfied (Satisfied) **Dissatisfied** **Very Dissatisfied**

2. **How did you originally learn about the NPCRSP?**

   I learned about the NPCRSP through Clark Atlanta University

3. **Did you have a clear set of expectations of the NPCRSP when you became a NPCR Scholar?**

   I had a clear set of expectation in terms of truly understanding what doing research is and learning the techniques use for it. The program met these expectations.

4. **How would you rate your research experience as it relates to helping you make decisions related to your education and career plans?**

   The program was very insightful in helping to sharp my career plans and knowing what are the steps I will need to take to reach those goals.

5. **What do you consider the main benefit(s) of the NPCRSP?**

The professional life experience is what I consider to the main benefit of the NPCRSP

6. **What suggestions would you give for the program for next summer?**

For next Summer I would suggest that the program makes better arrangement ~~for~~ as far as getting wages to students earlier.

2013 Nebraska Prostate Cancer Research Scholars Program

Student Evaluation

1. **How satisfied are you with the Nebraska Prostate Cancer Research Scholars Program(NPCRSP)?**

Very Satisfied **Satisfied** **Dissatisfied** **Very Dissatisfied**

2. **How did you originally learn about the NPCRSP?**

~~From~~ I learned about this program Marisha and Dr. Otero at Clark Atlanta University

3. **Did you have a clear set of expectations of the NPCRSP when you became a NPCR Scholar?** NO I did not

4. **How would you rate your research experience as it relates to helping you make decisions related to your education and career plans?**

It increased my interest in possibly working in prostate cancer rather than breast cancer, but it more so comfirmed my passion to pursue oncology

5. **What do you consider the main benefit(s) of the NPCRSP?**

I was able to gain my first lab research experience

6. **What suggestions would you give for the program for next summer?**

more ice breakers for CAU & INBRE program

2013 Nebraska Prostate Cancer Research Scholars Program

Student Evaluation

1. **How satisfied are you with the Nebraska Prostate Cancer Research Scholars Program(NPCRSP)?**

   Very Satisfied **Satisfied** **Dissatisfied** **Very Dissatisfied**

2. **How did you originally learn about the NPCRSP?**

   My home institution had an application process for a program in Nebraska and my genetics teacher.

3. **Did you have a clear set of expectations of the NPCRSP when you became a NPCR Scholar?**

4. **How would you rate your research experience as it relates to helping you make decisions related to your education and career plans?**

   The NPCRSP was very helpful. It provided networking opportunities and educational panels from a variety of science related professions to fully engage and encourage students (myself).

5. **What do you consider the main benefit(s) of the NPCRSP?**

6. **What suggestions would you give for the program for next summer?**

> more group discussions
-> more engagement or activities that bring together the INBRE scholars
-> more communication in terms of technical preparation (paperwork, checks)

2013 Nebraska Prostate Cancer Research Scholars Program

Student Evaluation

1. **How satisfied are you with the Nebraska Prostate Cancer Research Scholars Program(NPCRSP)?**

   Very Satisfied | **Satisfied** | **Dissatisfied** | **Very Dissatisfied**

2. **How did you originally learn about the NPCRSP?**

   I actually heard about this internship thro my institution and the mentors and faculty of this program. We had meet previously.

3. **Did you have a clear set of expectations of the NPCRSP when you became a NPCR Scholar?**

   Yes I had a clear set of expectations of the NPCRSP
   - informative, interactive learning
   - one-on-one based (post-doctoral mentor and PI mentor) (Lab environment)
   - Setup, orientation, and everything
   - It was different

4. **How would you rate your research experience as it relates to helping you make decisions related to your education and career plans?**

   I would rate this research experience an 8, and these seminars were very useful in deciding on current career trends.

5. What do you consider the main benefit(s) of the NPCRSP?

The main benefits

- building Lifelong relationships
- building networking opportunities and possible employment opportunit after graduation
- experiencing / Learning diversity & about scientific companies and lab techniques.
- Mentor experience – Mentee (labatory setting / professionalism

6. What suggestions would you give for the program for next summer?

MD/PHD
MD
MD/MBA

- More panel dsuwssions, a more diversity in career fields if you all can get a ~~career~~ career fair going where students speak with them or sum
- More interactive activities for incoming students probably have a mixer with old students interact with new students ~~no~~ a reunion.
- Better communication & having everything ready for summer students (everything including pay ~~day~~ roll and pay day)

Graduate
Poster #26

INTRACELLULAR MECHANISMS OF ARKADIA ABOLISHED TGF-β INDUCED PROTEASOME DEGRADATION OF SKI PROTEIN IN PROSTATE CANCER CELLS

BaoHan T Vo, **Maryam Boseman**, Chelesie Leath, Shawna Battle and Shafiq A. Khan, Center for Cancer Research and Therapeutic Development, Department of Biological Sciences, Clark Atlanta University, Atlanta, GA.

Transforming growth factor-β (TGF-β) pathway has been established as essential for cancer progression because of its prominent role in the regulation of cell growth, differentiation and

migration. The intracellular mechanisms involved in mediating the effects of TGF-β in prostate cancer during various stages of the disease are largely unknown. Previously we showed that Sloan Kettering-Institute (Ski) protein, a negative regulator of TGF-β signaling, is highly expressed in prostate cancer cells and tissues in contrast to normal cells. We also observed that TGF-β induced a rapid degradation of Ski protein mediated by the proteasomal pathway. Previous studies have indicated that the ubiquitin-proteasome pathway tightly regulates TGF-β signaling through E3 ubiquitin ligases that recognize and degrade target proteins. In this study, we determined the intracellular components of TGF-β signaling involved in Ski degradation in prostate cancer cells. Reverse transcriptase-polymerase chain reaction and Western Blot analyses were performed to determine the basal and TGF-β1-induced expression levels of selected E3 ubiquitin ligases in prostate cells. To determine whether knockdown of endogenous levels of Smurf1, Smurf2, Arkadia, or CHIP could abrogate TGF-β induced degradation of Ski, we performed siRNA transfection in DU145 and PC3 cells and treated them with TGF-β1. Western blot analysis confirmed a reduction in protein expression levels of Ski, Smurf1, Smurf2, Arkadia, and CHIP. We observed no significant differences in E3 ligase gene expression among the various cell lines. Protein levels of Smurf1, Smurf2 and CHIP were ubiquitously expressed in both normal and cancerous cells; however, the Arkadia protein was expressed higher in normal cells than in cancer cells. Treatment with TGF-β1 increased Smurf1, Smurf2, Arkadia, and CHIP at the mRNA and protein levels. Furthermore, knockdown of endogenous Arkadia abolished TGF-β induced degradation of Ski, indicating that Arkadia is specifically responsible for degrading Ski protein. These studies provide a better understanding on the intracellular mechanisms regulated by TGF-β in the prostate cells and show that the effects of TGF-β are mediated by Arkadia E3 ubiquitin ligases.

Acknowledgements: These studies were supported by the NIH/NCRR/RCMI grant #G12MD007590, NIH/NIMHD #5P20MD002285 and Title III Grant #22210K.

Immunocytochemistry. Chromatin Immunoprecipitation (ChIP) was performed with IgG, RNA polymerase, EZH2 and H3K27Me3 antibodies in both prostate cancer cell lines and prostate cancer tissue samples. IHC demonstrated decreased Id4 protein expression in human prostate tissue samples whereas higher nuclear Id4 expression was found in normal prostate tissues. Re-expression of Id4 in EZH2 silenced DU145 demonstrated that Id4 is regulated in an EZH2 dependent manner. ChIP data on prostate cancer samples and cell lines suggested EZH2 occupancy and H3K27Me3 marks in the Id4 promoter region. Thus, Id4 appears as a potential tumor suppressor gene that is epigenetically silenced during prostate cancer development. H3K27Me3 marks and EZH2 occupancy suggest a PRC2 dependent mechanism in Id4 promoter silencing in prostate cancer.

Acknowledgement: The research was supported by NIH/NCI-RO1CA128914 and in part by NIH/NCRR/RCMI G12RR03062.

Postdoc
Poster #51

Gai2 IS CRITICAL FOR TGFβ1, PGE2, OXT AND EGF INDUCED MIGRATION IN PROSTATE CANCER CELLS

HimaBindu Chunduri, BaoHan T. Vo, Chelesie Leath and Shafiq A. Khan, Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA.

Previously, our lab showed that Giα2 is essential for Oxytocin (OXT) and EGF induced migration in prostate cancer (PC3) cells. We also found that TGFβ1, PGE2, OXT and EGF induce migration in PC3 cells and these effects of TGFβ1, PGE2 on cell migration are mediated via PI3K/AKT/mTOR pathway. The current study was aimed to understand how PI3K signaling and GPCR signaling pathways interact in response to these ligands in PC3 cell migration. PC3 cells treated with TGFβ1, PGE2, OXT and EGF were used for transwell cell migration assay and p-AKT activation analysis. Pertussis toxin (PTX) is Gi protein inhibitor. The cells were preincubated with PTX (100ng/ml) for 24 h and treated with TGFβ1, PGE2, OXT and EGF. These cells were analyzed for activation of PI3K/AKT pathway and cell migration. We also studied the effects of knockdown of endogenous Giα2 on TGFβ1, PGE2, OXT and EGF induced cell migration and AKT phosphorylation. There was an increase in cell migration and AKT phosphorylation with TGFβ1, PGE2, OXT and EGF. An inhibitor of PI3K (LY294002) blocked migration and AKT phosphorylation in response to OXT. PTX blocked the effects of TGFβ1, PGE2 and OXT on cell migration and AKT phosphorylation but not blocked the effects of EGF. However, Giα2 knockdown blocked migration in all TGFβ1, PGE2, OXT including EGF induced cells. Based on these results we are suggesting that Giα2 plays a critical role in migration in response to these four ligands.

Acknowledgements: These studies were supported by the NIH/NIMHD/RCMI grant #G12MD007590, NIH/NIMHD #5P20MD002285.

Undergraduate
Poster #4

DIFFERENTIAL ACTIVITY IN THE PARAVENTRICULAR NUCLEUS NADPH –D CELLS WITHIN THE HYPOTHALAMUS

Jeremy Cobbs-Hart[1], Mary Inez Smith[1], India Nichols[1], Lindsey Crawford[1], C. O. Okere[1], Clark Atlanta University, [1]Department of Biological Sciences, [2]Department of Psychology, Atlanta, GA.

Our goal is to identify the activity and topography of NADPH-d cells in the paraventricular nucleus (PVN) of the rat brain. The PVN is an area of magnocellular neuro-secretory cells in the hypothalamus

Graduate
Poster #31

INHIBITION OF CONSTITUTIVE ARYL HYDROCARBON RECEPTOR (AHR) SIGNALING ATTENUATES ANDROGEN INDEPENDENT SIGNALING AND GROWTH IN (C4-2) PROSTATE CANCER CELLS

Maryam Ghotbaddini, Cindy Tran, Oliver Richmond, LaTayia Aaron, Joann Brooks Powell, Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA.

The aryl hydrocarbon receptor (AhR) is a member of the basic-helix-loop-helix family of transcription factors. AhR is widely known for regulating the transcription of drug metabolizing enzymes involved in the xenobiotic metabolism of carcinogens and therapeutic agents, such as cytochrome P450-1B1

(CYP1B1). Additionally, AhR has also been reported to interact with multiple signaling pathways during prostate development. Here we investigate the effect of sustained AhR signaling on androgen receptor function in prostate cancer cells. Immunoblot analysis shows that AhR expression is increased in androgen independent (C4-2) prostate cancer cells when compared to androgen sensitive (LNCaP) cells. q RT-PCR studies revealed constitutive AhR signaling in C4-2 cells without the ligand induced activation required in LNCaP cells. A reduction of AhR activity by short RNA mediated silencing in C4-2 cells reduced expression of both AhR and androgen responsive genes. The decrease in androgen responsive genes correlates to a decrease in phosphorylated androgen receptor and androgen receptor expression in the nucleus. These data indicates that AhR is required to maintain hormone independent signaling by the androgen receptor in C4-2 cells. Collectively, these data provide evidence of a direct role for AhR in androgen independent signaling and provides insight into the molecular mechanisms responsible for sustained androgen receptor signaling in hormone refractory prostate cancer.

Acknowledgements: Grant # 8 G12 MD007590-NIH/National Institute on Minority Health and Health Disparities (NIMHD) Grant # 5 G12 RR003062.

Undergraduate
Poster #13

INHIBITING RAC 1- GTPase ACTIVITY IN OVARIAN CANCER CELLS

Chelesie Leath, Chao Jiang, John Davis and Shafiq Khan, Department of Obstetrics and Gynecology Research, University of Nebraska Medical Center, Omaha, Nebraska; Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA.

Rho proteins have been described as "molecular switches" and play a role in many common cellular functions such as cell proliferation and survival, apoptosis and gene expression, but also in a number of diseases, neurological disorders and different types of cancer. Because of their multifunctional properties, these proteins are thought to be ideal candidate for anti-cancer therapeutics and drug development. However, the mechanisms involved in regulating Rho GTPase activity are still largely unknown. To date, most efforts focused on inhibitors that block indirectly, by targeting enzymes involved in post-translational processing or downstream protein kinase effectors. Identifying small molecular inhibitors to directly target RHO GTPase functions may be ideal for inhibiting progression

of aggressive cancer into more advanced stages. Preliminary studies show that a well studied Rho GTPase, RAC-1 and its splice variant RAC 1B are highly expressed in ovarian cancer cells. Human ovarian surface epithelial cell, Hose 969 and T80 and ovarian cancer cells, IGROV-1, SKOV3, TOV21G, were treated for 24 and 48 hours with different concentrations of NSC23766 and EHT 1864. MTT assay revealed a decrease in viability of both epithelial and ovarian cancer cells. The purpose study is to determine the effect of small molecular inhibitors NSC23766 and EHT 1864 on human ovarian surface epithelial and ovarian cancer cells, in the presence and absence of anti-cancer drugs, as a potential therapy approach.

Acknowledgements: These studies were supported by the NIH/NIMHD/RCMI grant #G12MD007590, NIH/NIMHD #5P20MD002285.

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Acknowledgements: These studies were supported by the NIH/NIMHD/RCMI grant #G12MD007590, NIH/NIMHD #5P20MD002285.

Undergraduate
Poster #14

SNAIL TRANSCRIPTION FACTOR REGULATES CATHEPSIN L AND MASPIN IN PROSTATE CANCER CELLS

Quentin Loyd, Bethany N. Smith, Veronica Henderson, Danielle McKeithen, Marisha Morris, Peri Nagappan, and Valerie A. Odero-Marah, Department of Biological Sciences and the Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA.

Snail transcription factor is increased in prostate cancer and has a role in metastasis. Snail promotes epithelial-mesenchymal transition (EMT) by decreasing adhesion and increasing migration and invasion. Snail functions by binding to and repressing tumor suppressors such as maspin. Snail activity is associated with degradation of the extracellular matrix, but the mechanism(s) for this is not clear. Cathepsin L is a serine protease that degrades components of the extracellular matrix (ECM) when activated. Cathepsin L activity is associated with tumor metastasis, invasion, and bone turnover. We hypothesized that high levels of Snail will influence the localization and levels of Cathepsin L and maspin. We used an established prostate cancer progression model for our studies; LNCaP prostate cancer cells overexpressing Snail gene (LNCaP Snail18) or LNCaP cells with empty vector (LNCaP Neo5) as a control. Preliminary data indicates that Snail was more highly expressed in the LNCaP Snail 18 clone compared to the control. We also found that LNCaP Snail 18 had higher migration compared to LNCaP Neo 5 using collagen type I ECM. To determine if this change in behavior was due to an interaction between Snail and Cathepsin L during prostate cancer progression, we injected $2x10^6$ LNCaP Neo 5 or LNCaP Snail 18 into immunocompromised male mice subcutaneously. After 12 weeks, mice were sacrificed; the tumor xenograft tissues were excised, fixed, sectioned and stained for expression of Snail, maspin and Cathepsin L using immunofluorescence technique. We observed that Snail and Cathepsin L staining was higher in LNCaP Snail 18 tissue but maspin was lower. We found that Snail, maspin and Cathepsin L were more localized in the cytoplasm of LNCaP Neo 5 xenografts, but Snail and Cathepsin L were more nuclear in LNCaP Snail 18 xenografts. We concluded that Snail may regulate prostate cancer progression by increasing the production of Cathepsin L while decreasing maspin in order to migrate and invade. These studies begin to uncover the interactions between Snail and its downstream targets during prostate cancer progression.

Acknowledgements: These studies were supported by grants NIH/NCRR/RCMI grant #G12RR003062-22, NIH 1 R15 grant #CA169899-01A1 and NIH/NIGMS RISE Grant #5R25GM060414.

Undergraduate
Poster #15

NOVEL IMIDAZOPYRIDINE DERIVATIVES INHIBIT ANDROGEN-INDEPENDENT PROSTATE CANCER CELL PROLIFERATION

Marisha Morris[1], Sakthivel Muniyan[2], Jennifer G. Dwyer[2], Xiu Bu[3], Ming-Fong Lin[2] Department of Biology, Clark Atlanta University, Atlanta, GA Department of Biochemistry & Molecular Biology, University of Nebraska Medical Center, Omaha, NE Department of Chemistry, Clark Atlanta University, Atlanta, GA.

In the present study, we determined the antiproliferative efficacy of novel imidazopyridine derivatives in androgen-independent prostate cancer cell lines: LNCaP C-81 and MDA PCa2b AI PCa cells. Our preliminary cell growth analysis showed that at least three of these novel derivatives significantly inhibited the proliferation in both the cell lines examined. Further Western blot analyses confirm the PCa cell growth inhibition with a significant reduction of cell cycle proteins cyclin D1, cyclin B1 and PCNA. Further, in all the treated groups, PCa cell growth inhibition is accompanied by a significant reduction of either pAkt and/or pStat5 inhibition. Collectively, the present data suggest that these imidazopyridine derivatives exhibit potential anticancer activity in PCa by targeting both PIK3/Akt and pStat5 pathway.

Acknowledgement: This research was supported by funding from DOD grant # PCPC121645.

Graduate
Poster #42

ID4 ACTS AS A TUMOR SUPPRESSOR BY REGULATING THE TRANSCRIPTIONAL ACTIVITY OF p53

Derrick Morton, Ashley Evans Knowell, Divya Patel, Pankaj Sharma, Shanora Glymph Brown and Jaideep Chaudhary, Department of Biological Sciences, Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA.

The physiological mechanisms that can restore biological activity of mutant p53 is an area of high interest given that mutant p53 expression is observed in one third of prostate cancer and more than 50% of all cancers. Here we demonstrate that Id4 (inhibitor of differentiation-4) a dominant negative regulator of bHLH transcription factors can restore the mutant p53 transcriptional activity in prostate cancer cells. Id4 is highly expressed in the normal prostate and decreased in prostate cancer due to promoter hypermethylation. Prostate cancer cell lines: DU145 harbors mutant p53 also lack Id4 expression, and LNCaP cells with wild type p53 express Id4, whereas PC3 cells are null for p53 and express low levels of Id4. The p53 mutants (P223L and V274F) in DU145 cells are within the DNA binding domain and abrogate p53 transcriptional activity due structural de-stabilization and/or DNA interactions. Ectopic expression of Id4 in DU145 cells resulted in increased apoptosis and expression of BAX, PUMA and p21, the transcriptional targets of p53. DNA binding, p53 luciferase reporter studies and ChIP analysis demonstrated that mutant p53 gains Id4 dependent DNA binding and transcriptional activity in part due to CBP/p300 dependent acetylation of p53 at lysine 373. Loss of Id4 in LNCaP cells also abrogated wild type p53 DNA binding and transcriptional activity with concomitant loss of CBP/p300 requirement and decreased acetylation of p53. To further elucidate Id4 dependent restoration of biological activity of p53 we stably transfected p53-null cell line PC3, which has endogenous Id4 with mutant p53 mimicking DU145 cells. mRNA, protein levels and apoptosis assays were used to determine the effects on cell death and to determine if mutant p53 had the ability to transactivate upstream/downstream targets (MDM2, PUMA, and p21). Our results indicate that

Acknowledgements: This research is supported by National Center on Minority Health and Health Disparities (NCMHD) grant MD003399-04.

Undergraduate
Poster #20

NON-MUSCLE MYOSIN IIA-MEDIATED GOLGI FRAGMENTATION IN ADVANCED PROSTATE CANCER CELLS

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Prostate cancer is the second leading cause of cancer death among men older than 55 in the United States. Androgen ablation is a main therapy for prostate cancer. However, androgen refractory will develop within few years. Once it occurs, the cancer is incurable. Therefore, there is a pressing need to understand the biology of the hormone refractory prostate cancer to aid the development of therapy for these patients. Recently, we showed that the Golgi was fragmented in both colon cancer tumors as well as metastatic HT29 cells. Under circumstances inducing stress on the cell, the Golgi is known to undergo morphological changes and fragmentation[5]. Recently, it was reported that non-muscle myosin IIA (NMIIA) was associated with the Golgi stacks[4,12] and Golgi glycosyltransferases (GT) and not matrix proteins are the binding partners of NMIIA[10] The fragmented Golgi was reversed to compact morphology after treatment with a non-muscle myosin IIA inhibitor Blebbistatin. We detected that expression of Golgi matrix protein, Giantin in androgen-refractory DU145 and PC-3 cells was substantially lower than in LNCap cells. Next, we treated cells with 30 μM of NMIIA inhibitor Blebbistatin for 16 h; corresponding amount of DMSO was used as a control. Cells were subjected to immuno-staining with Giantin. The goal of this study was to determine if Golgi fragmentation occurs in prostate cancer cells. If it does, it can be reversed to compact morphology by treatment with Blebbistatin. By confocal immunofluorescence microscopy, we showed that LNCaP cells, which are sensitive to androgens, have compact morphology while DU145 and PC3 cells, which are androgen-refractory and more tumorigenic than LNCaP cells, have fragmented Golgi. Treatment of these two cells with Blebbistatin reversed the Golgi to compact morphology. We conclude that the Golgi apparatus in aggressive prostate cancer cells is fragmented and this phenomenon is mediated by non-muscle myosin IIA. We plan to determine whether Golgi fragmentation also occurs in advanced prostate tumors and whether restoration of the normal Golgi morphology in prostate cancer cells corrects glycosylation abnormality.

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Antiproliferative activity of novel imidazopyridine derivatives on castration-resistant human prostate cancer cells

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ABSTRACT

Metastatic prostate cancer (mPCa) relapses after a short period of androgen deprivation therapy and becomes the castration-resistant prostate cancer (CR PCa); to which the treatment is limited. Hence, it is imperative to identify novel therapeutic agents towards this patient population. In the present study, antiproliferative activities of novel imidazopyridines were compared. Among three derivatives, PHE, AMD and AMN, examined, AMD showed the highest inhibitory activity on LNCaP C-81 cell proliferation, following dose- and time-dependent manner. Additionally, AMD exhibited significant antiproliferative effect against a panel of PCa cells, but not normal prostate epithelial cells. Further, when compared to AMD, its derivative DME showed higher inhibitory activities on PCa cell proliferation, clonogenic potential and *in vitro* tumorigenicity. The inhibitory activity was apparently in part due to the induction of apoptosis. Mechanistic studies indicate that AMD and DME treatments inhibited both AR and PI3K/Akt signaling. The results suggest that better understanding of inhibitory mechanisms of AMD and DME could help design novel therapeutic agents for improving the treatment of CR PCa.

Introduction

Prostate cancer (PCa) is the second leading cause of cancer deaths in United States men [1]. Androgen-deprivation therapy (ADT) has been the mainstay of treatment towards patients with metastatic PCa [2,3]. Although most of PCa respond well to ADT initially, most PCa relapse and become the castration-resistant (CR) PCa [2,3]. CR PCa is lethal with about 18-month median survival time [4]. Currently, chemotherapy is the standard-of-care treatment for CR PCa. Nevertheless, it only provides a minimal improvement in survival. Hence, the prime need is to identify a novel therapeutic agent to improve the efficacy of CR PCa treatment.

Imidazopyridine derivatives are a class of novel compounds which have aromatic aldehydes and a pyridine group, and possess medicinal importance [5–7]. Recent studies show imidazopyridine derivatives exhibit potent antitumor activity against breast and pancreatic cancers [8,9]. Nevertheless, no report is currently available on the antiproliferative effect of imidazopyridine derivatives on CR PCa. Therefore, the present study is undertaken to synthesize a series of novel imidazopyridine derivatives and to investigate their antiproliferative effect against a panel of PCa cancer cell lines including both AR-positive and AR-negative AI PCa cells which

Abbreviations: ADT, androgen deprivation therapy; AI, androgen-independent; AR, androgen receptor; AS, androgen-sensitive; CR PCa, castration-resistant prostate cancer; DHT, dihyrotestosterone; ECL, enhanced chemiluminescence; FBS, fetal bovine serum; PCa, prostate cancer; PI3K, phosphatidylinositol-3 kinase; PSA, prostate-specific antigen; p66Shc, a 66 kDa Src homologous-collagen homologue; SR, steroid-reduced.

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exhibit diverse phenotypes of CR PCa. Our results show that imidazopyridine derivatives inhibit CR PCa cell proliferation, decrease migration and *in vitro* tumorigenicity. Our data, to the best of our knowledge, is the first report that clearly shows the potential of this family of compounds to serve as effective molecules towards CR PCa treatment by inhibiting AR and PI3K/Akt signaling.

Materials and methods

Materials

RPMI 1640, Keratinocyte SFM medium, gentamicin, and L-glutamine were from Invitrogen (Carlsbad, CA, USA). Fetal bovine serum (FBS) and charcoal/dextran-treated FBS were purchased from Atlanta Biologicals (Lawrenceville, GA, USA). Polyclonal antibodies (Abs) recognizing all three isoforms of Shc protein were obtained from Upstate (Lake Placid, NY, USA). Anti-cyclin B1, anti-cyclin D1, anti-AR, anti-Bax, anti-Bcl_{XL}, anti-PCNA, anti-p53, anti-PSA and horseradish peroxidase-conjugated anti-mouse, anti-rabbit, anti-goat IgG Abs and Akt inhibitor (MK2206) were all from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phospho-Akt (Ser473) and anti-Akt Abs were from Cell Signaling Technology (Beverly, MA, USA). Anti-β-actin Ab and DHT were obtained from Sigma (St. Louis, MO, USA). PI3K inhibitor (LY294002) was obtained from Calbiochem (San Diego, CA, USA).

Synthesis of imidazopyridines

The synthesis of the imidazopyridine compounds were essentially followed the protocol described in our previous publication [7]. All the reactions were performed in flame-dried glassware under the nitrogen environment using freshly diluted solvents. All the chemicals and solvents were used as received. 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded with TMS as an internal standard for reference. The C, H, and N contents were obtained through combustion analysis. Melting points are uncorrected. The compounds were synthesized using a mixture consisting of di-2-pyridyl ketone, substituted aromatic aldehydes and ammonium acetate in 35 mL of glacial acetic acid [7]. Briefly, phenol, 4-actetamido-benzaldehyde, benzenamine and N-N-dimethyl aniline were used as substituted aldehydes to synthesize IMP-PHE, -AMN, -AMD and -DME, respectively (Fig. 1). The reaction was stirred at 110 °C under N_2 and was monitored by TLC (EtOAc:Hex = 1:1) alumina plates. Upon completion, the reaction was allowed to cool to room temperature and poured into 200 mL of ice water. The yielded solid was filtered, dried, and recrystallized with appropriate solvent to obtain an analytically pure compound [7].

Cell culture

Human prostate carcinoma cell lines LNCaP, MDA PCa2b, PC-3, DU 145 and immortalized normal prostate epithelial cells RWPE1 and PZHPV-7 cells were all obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained as described [10,11]. LNCaP, PC-3 and DU 145 cells were routinely maintained in the regular culture medium, i.e., phenol red-positive RPMI 1640 medium supplemented with 5% FBS, 2 mM glutamine and 50 μg/mL gentamicin. LNCaP C-81 cells were described previously [12,13], and exhibit the CR phenotype including functional AR expression and prostate-specific antigen (PSA) secretion with rapid cell growth in the steroid-deprived condition [12,13]. Further, those cells exhibit the intracrine regulatory mechanism [14]. Similarly, MDA PCa2b-AI cells exhibit androgen-independent (AI) proliferation and were obtained as described [10,15,16]. MDA PCa2b-AI cells were cultured in BRFF-HPC1 medium containing 20% FBS, 2 mM glutamine and 50 μg/mL gentamicin [10,16]. RWPE1 and PZHPV-7 cells were grown in Keratinocyte-SFM supplemented with bovine pituitary extract (25 μg/mL) and recombinant epidermal growth factor (0.15 μg/mL) containing 50 μg/mL gentamicin.

For steroid-starvation, cells were maintained in steroid-reduced (SR) media, i.e., phenol red-free RPMI 1640 medium containing 5% heat-inactivated, charcoal/dextran-treated FBS (v/v), 2 mM glutamine, 50 μg/mL gentamicin and 1 nM 5α-dihydrotestosterone (DHT), which mimics the condition of androgen ablation treatment in patients. PHE, AMN, AMD and DME were dissolved in DMSO as 1000 × concentrated stock solutions, stored at −20 °C, and diluted in the respective culture media at the time of use. Control cells were treated with media containing an equal amount of DMSO.

Cell proliferation assay

For the cell proliferation assay in regular media, LNCaP C-81 cells were plated at a density of 2×10^3 cells/cm^2 in 6-well plates for 3 days and then treated with 10 μM of PHE, AMN or AMD in fresh media for 3 days. To compare the effect of AMD on different prostate cells, PCa cells and immortalized normal prostate epithelia were plated in their respective regular media at the described cell density for 3 days, followed by the addition of 10 μM AMD in fresh media. The cell numbers were counted using a Cellometer Auto T4 Image-based cell counter (Nexcelom Bioscience), which cell number was counted by Tryphan blue exclusion assay.

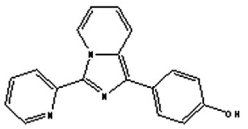
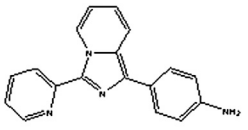
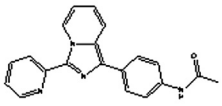
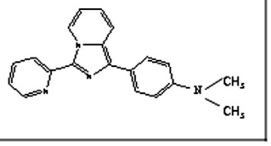
Name of the compound	Formula Weight	Structure
IMP-PHE	287.13	
IMP-AMD	286.33	
IMP-AMN	328.37	
IMP-DME	314.38	

Fig. 1. The structure of imidazopyridine derivatives.

The ratio of live cell number in the experimental group to that of the control group was calculated for indicating cell proliferation.

To determine the effect of AMD and DME in SR media, C-81 cells were plated in regular medium for 3 days and then steroid-starved for 2 days in a SR medium. After being fed with fresh SR medium, cells were treated with different concentrations of AMD or DME. Control cells were treated with solvent alone. The cell numbers were counted and the ratio of cell growth was calculated as described above.

Kinetics of cell growth determination

To determine the time-dependent effect of AMD and DME on the growth of LNCaP C-81 cells, cells were seeded on six-well culture plates with triplicates and maintained in regular culture medium for 3 days. For SR condition, cells were maintained for additional 48 h in SR medium. One set of attached cells from each culture condition was harvested and counted as day 0 as described above. The remaining attached cells were fed with fresh regular or SR medium containing solvent, AMD or DME, and harvested on days 1, 3 and 5 for total cell number counting. The fresh medium was added to the remaining LNCaP cells on days 1 and 3.

Immunoblot analysis

To analyze cellular proteins, cells were harvested by scraping. The cell pellet was rinsed with ice-cold 20 mM HEPES-buffered saline (pH 7.0), lysed in ice-cold cell lysis buffer containing protease and phosphatase inhibitors, and the total lysate protein was prepared accordingly [12,17]. An aliquot of total cellular lysate proteins was electrophoresed on SDS–polyacrylamide gels (7.5–12% acrylamide) for western blot analyses [12]. The proteins of interest were visualized by an ECL detection system. β-actin was detected as a loading control.

Cell migration

Cell migration was determined using an *in vitro* 'wound-healing' assay. Briefly, PC-3 cells were seeded in 6-well plates and grown for 48 h to reach confluence. Wounds were made in the confluent cell monolayer using a sterilized P200 pipette tip. The wound was washed with RPMI medium without FBS to remove all the detached cell debris. Remaining cells were treated with fresh media, media containing 10 μM AMD or DME. After 12 h, cells were stained with crystal violet and images were taken. The wound gap was measured, and % wound healing was calculated. The average % of wound healing was determined by at least 3 measurements per scratch from 2 independent experiments.

Clonogenic cell growth assay and anchorage-independent soft agar assay

The clonogenic cell growth was described previously [15,18]. Briefly, LNCaP C-81 cells were plated in regular culture medium at densities of 20, 200, and 2000 cells per well in 6-well plates. After overnight incubation, unattached cells

were removed and attached cells were fed with fresh regular medium with or without respective compounds. 2 ml of fresh medium containing respective compound were added into each well on days 5 and 8. On day 10, the attached cells were stained with 0.2% crystal violet solution containing 50% methanol.

The anchorage-independent growth of cells was determined by soft agar analysis with modifications [18]. Briefly, 5×10^4 cells were seeded in 0.25% agarose on the top of a base layer containing 0.3% agarose. One day after seeding, cell clumps containing more than one cell were excluded from the study, and the cells were fed with fresh medium containing respective treatment compounds once in every 3 days. The colony number was counted after 4 weeks of incubation at 37 °C. Alternatively, the colonies were stained with 0.1% crystal violet solution containing 20% methanol and counted.

Statistical analysis

Each set of experiments was performed in duplicate or triplicate, as specified in the figure legend or experimental design, repeated at least two or three times as independent experiments. The mean and standard error values of experimental results were calculated. A student-*t* test was used for comparison between each group. $p < 0.05$ was considered statistically significant [18]. The relationship between the relative ratios of cell proliferation and the dosage of compounds was evaluated by their correlation coefficient (r). The correlation was considered significant when the p-value was less than 0.05.

Results

Anti-proliferative effect of imidazopyridine derivatives on LNCaP C-81 PCa cells

To determine the anti-proliferative effect of imidazopyridine derivatives, a cell proliferation assay was initially performed in LNCaP C-81 PCa cells because these cells exhibit many biochemical properties of the advanced CR PCa phenotype [12–14]. After 72 h treatment with 10 μM imidazopyridine derivatives in the regular cell culture medium, all three compounds PHE, AMD and AMN reduced cell proliferation by an average of 42%, 53% and 18%, respectively (Fig. 2A). The antiproliferative activity of these compounds respectively correlated with decreased protein levels of proliferation markers cyclin B1, cyclin D1 and PCNA in those cells (Fig. 2B). In AMD-treated LNCaP C-81 cells, AR protein level was also decreased and tumor suppressor p53 and proapoptotic protein Bax were elevated (Fig. 2B). Since AMD exhibited the highest inhibitory activity among the three compounds examined, AMD compound was used for further experiments.

Dose- and time-dependent effects of AMD on LNCaP C-81 cells

We examined the dose-dependent effect of AMD on LNCaP C-81 cells. Upon treated with 0–10 μM AMD for 72 h, cell growth was analyzed. In regular culture media, AMD inhibited LNCaP C-81 cell growth in a dose-dependent manner (Fig. 2C). 10 μM AMD inhibited 54% cell growth and were used in future experiments.

To determine the kinetic effect of AMD, at each time point, cells in triplicates from each group were harvested and cell number determined. The remaining cells were fed with fresh media or media plus AMD. As shown in Fig. 2D, after 24-h treatment, cell growth was decreased by about 19%. Upon 72-h treatment, approximately 53% growth inhibition was observed. Five-day treatment with 10 μM AMD inhibited the cell growth by over 60% (Fig. 2D). Thus, AMD suppressed LNCaP C-81 cell growth following a time course.

Antiproliferative effect of AMD on AR-positive and AR-negative PCa cells in comparison with immortalized normal prostate epithelial cells

The growth suppressive efficacy of 10 μM AMD was evaluated in a panel of AI cells including both AR-positive (LNCaP C-81 and MDA PCa2b AI) and AR-negative (PC-3 and DU145) PCa cells. Significant growth inhibition on all PCa cells tested was observed at 10 μM AMD (Fig. 2E). Notably, 10 μM AMD had only approximately 10% effect on the proliferation of immortalized normal prostate epithelial RWPE1 and PZHPV-7 cells (Fig. 2E). Together, our results showed that AMD exhibits potent inhibitory activity on AI PCa cell growth, but not normal prostate epithelia.

Comparative effects of AMD and DME in LNCaP C-81 cells

To improve the efficacy of AMD on inhibiting AI PCa cell growth, the amino functional group of AMD was modified and a new derivative DME was obtained (Fig. 1). Preliminary experiments revealed that 10 μM DME of 72 h-treatment decreased LNCaP C-81 cell proliferation by up to 88% ($p < 0.001$ vs. control, data not shown); while AMD inhibited cell growth by about 55% (data not shown and Fig. 2A). We then examined the dose-dependent effect of DME on LNCaP C-81 cells for 72 h. Our results showed both AMD and DME inhibit cell growth in a dose-dependent manner (Fig. 3A vs. 2C). In regular culture media, the efficacy of growth inhibition by DME was about two fold that of AMD (Fig. 3) with IC50 value of about 3.9 μM and 7.5 μM, respectively.

We analyzed the effect of AMD and DME in a SR medium containing 1 nM DHT, which mimics the clinical condition under androgen deprivation treatment. The same trend of dose-dependent inhibition was observed despite the inhibitory activity was decreased (Fig. 3B). In SR conditions, DME inhibited cell growth with IC_{50} of 6.3 μM, comparing with 11.4 μM of AMD. We thus performed further studies on AMD and DME at 10 μM in SR condition for clinical relevance.

Effects of AMD and DME on cell migration and in vitro tumorigenicity

The effects of AMD and DME on PCa cell migration were assessed by a wound healing assay with PC-3 cells as the model system since C-81 cells exhibit slow-migration (data not shown). As shown in Fig. 4A, both AMD and DME significantly inhibited PC-3 cell migration. After 12 h, 50–60% of the wound area remained open in AMD- and DME-treated cells, while the wound in solvent-treated control group was completely closed ($p < 0.01$ with control).

To explore the anti-tumorigenic potential of AMD and DME, a clonogenic assay was performed on LNCaP C-81 cells. As shown in Fig. 4B, after 10 day-treatment, both AMD and DME inhibited clonogenic growth. In comparison, DME inhibited clonogenic growth at all cell densities examined, while AMD had less inhibitory activity with colonies clearly seen in 2000 cells/well. Nevertheless, the size of AMD-treated colony was dramatically reduced, much smaller than controls.

An *in vitro* anchorage-independent soft agar assay was performed on LNCaP C-81 cells. As shown in Fig. 4C and D, after 4-week cultured at a density of 5000 cells per 35 mm dish there was visible colony formation in solvent-treated control groups. AMD treatment decreased the soft agar colony number by about 50%, and DME treatment inhibited over 90% (Fig. 4D). Further, both AMD and DME greatly reduced the size of colonies. Therefore, both AMD and DME exhibit anti-tumorigenic effects on both AI PC-3 and LNCaP C-81 cells (Fig. 4).

Kinetics of AMD and DME effects on LNCaP C-81 cell proliferation in SR condition

We investigated the kinetics of AMD and DME on LNCaP C-81 cell proliferation in SR conditions. As shown in Fig. 5A, after 3-day treatment in SR conditions, both AMD and DME significantly inhibited the growth of LNCaP C-81 cells, and DME exhibited a better efficacy than AMD. It should be noted that in SR conditions,

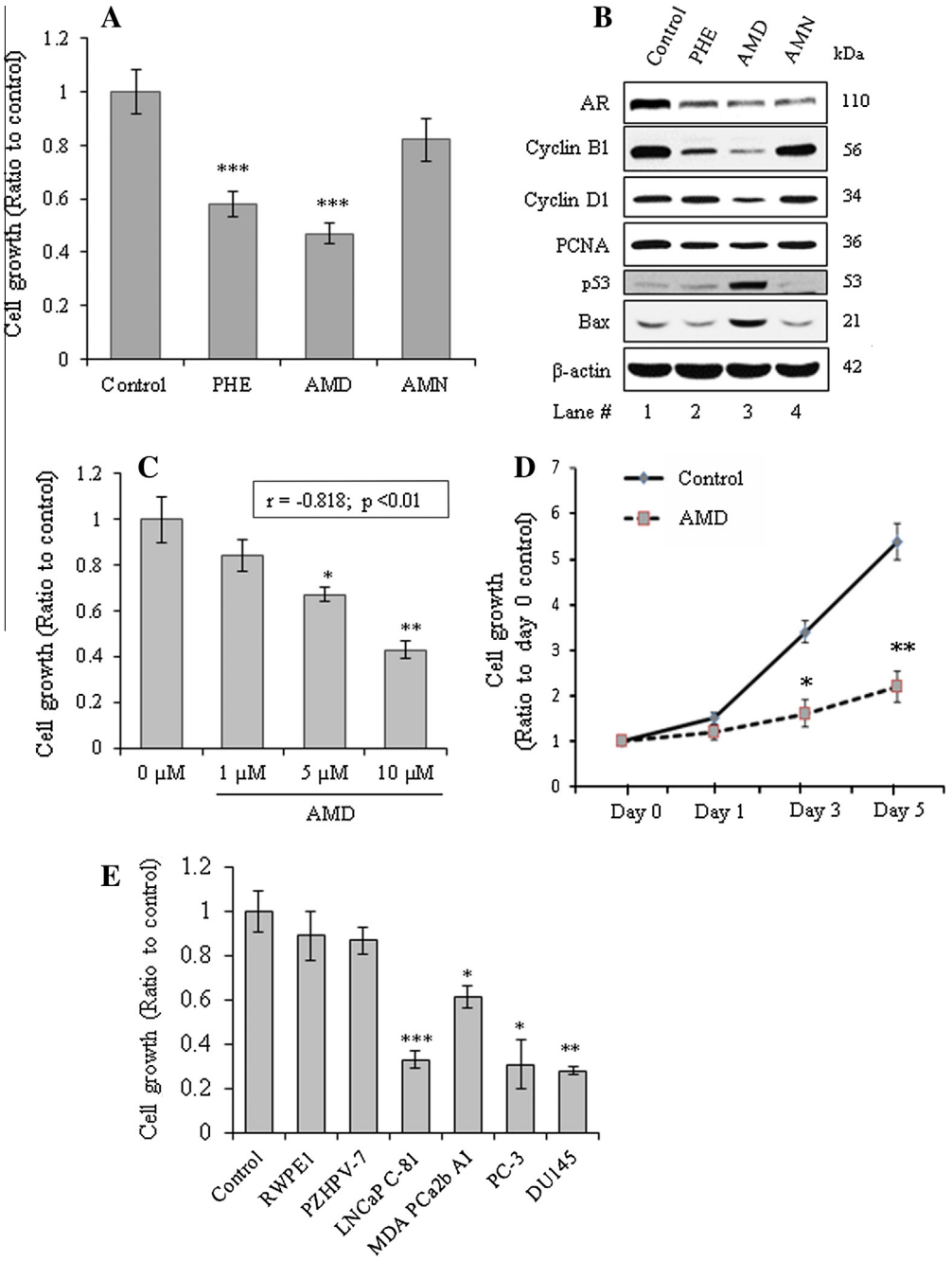


Fig. 2. Effects of imidazopyridine derivatives on the growth of PCa cells in regular culture conditions. A. LNCaP C-81 cells were plated in six-well plates at 2×10^3 cells/cm^2 in regular medium for 3 days, then 10 μM each of imidazopyridine derivatives (PHE, AMN and AMD) in fresh medium were added for 72 h. Cells were trypsinized and counted for live cell number. The results presented were mean ± SD; $n = 2 \times 3$. $^{***}p < 0.001$. B. Total cell lysate proteins from imidazopyridine derivatives-treated C-81 cells were analyzed for AR, p53, Bax, cyclin B1, cyclin D1, PCNA proteins. β-actin protein level was analyzed and used as a loading control. C. The dose-dependent effect of AMD on LNCaP C-81 cell growth in regular culture medium. Cells were plated in six-well plates at 2×10^3 cells/cm^2 in regular medium for 3 days and then treated with different concentrations of AMD in fresh medium. After 3 days, cells were trypsinized and counted for cell growth. The results presented were mean ± SD; $n = 2 \times 3$. $^{*}p < 0.05$; $^{**}p < 0.01$. D. Time-dependent effect of AMD on LNCaP C-81 cell growth in regular culture condition. Cells were plated in six-well plates at 2×10^3 cells/cm^2 in regular medium for three days, then 10 μM AMD were added. One set of cells in triplicates was harvested after 1, 3 and 5 days, and counted for cell growth. The fresh medium was added to remaining cells on days 1 and 3. The results presented were mean ± SE; $n = 2 \times 3$. $^{*}p < 0.05$; $^{**}p < 0.01$. E. Effects of AMD on the growth of various PCa cells and immortalized prostate epithelial cells. All cells were plated in six-well plates at the noted density in their respective medium for 3 days, then fresh media plus 10 μM AMD were added. After 3 days cells were trypsinized and counted for cell number. The results presented were mean ± SE; $n = 2 \times 3$. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. RWPEI – 4×10^3 cells/cm^2; PZ-HPV-7 – 6×10^3 cells/cm^2, LNCaP C-81 – 2×10^3 cells/cm^2; MDA PCa2b AI – 3×10^3 cells/cm^2; PC-3 – 2×10^3 cells/cm^2; DU145 – 2×10^3 cells/cm^2.

while control cells continuously grew on days 3 and 5, the cell number of AMD- and DME-treatment declined. Upon 5-day DME-treatment, the cell number was even lower than the pre-treatment day 0 cell number. The decrease in cell growth was reflected by cyclin D1 protein level (Fig. 5B).

Effects of AMD and DME on AR and Shc level in SR conditions

Since the majority of CR PCa cells still express functional AR for their growth requirement [19], we analyzed AR protein level in AMD- and DME-treated LNCaP C-81 cells under SR condition.

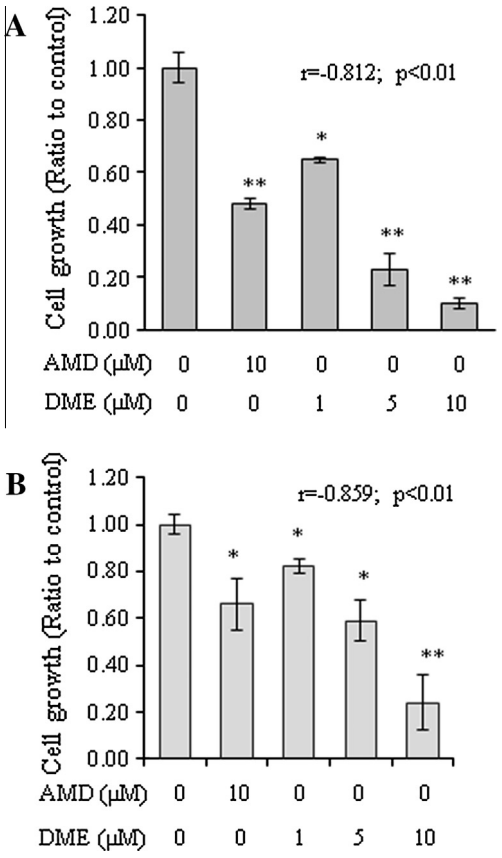


Fig. 3. Effects of AMD and DME on LNCaP C-81 cell proliferation in regular and steroid-reduced culture conditions. A. The effects of AMD and DME on LNCaP C-81 cell growth in regular culture medium. Cells were plated in six-well plates at 2×10^3 cells/cm^2 in regular medium for 3 days and then treated with 10 μM of AMD and different doses of DME in fresh medium for 3 days. B. The effects of AMD and DME on LNCaP C-81 cells in steroid-reduced condition. Cells were plated in six-well plates at 3×10^3 cells/cm^2 in regular medium for 3 days, followed by maintained in steroid-reduced media containing 1 nM DHT for 2 days. AMD and DME at different concentrations in fresh SR media containing 1 nM DHT were then added. After 3 days of treatment, cells were trypsinized and counted for cell numbers. The results presented were mean ± SD; $n = 2 \times 3$. $^{*}p < 0.05$; $^{**}p < 0.01$.

Fig. 5B showed AR protein level was greatly reduced in both AMD- and DME-treated cells at day 3 and day 5 after an initial elevation in day 1. Concurrently, the cellular level of PSA, an androgen-regulated protein, was closely associated with AR protein level. Thus, AMD- and DME-treatments decrease AR signaling.

The protein level of p66Shc, a 66 kDa Src homologous-collagen homologue (Shc), is involved in regulating the proliferation of several carcinoma cells including PCa cells and can be up-regulated by steroids [20–23]. Fig. 5B showed the protein level of p66Shc but not p52Shc or p46Shc was decreased in 3-day and 5-day AMD- and DME-treated cells. The decreased p66Shc protein level correlates with diminished cell proliferation, AR and cyclin D1 protein levels.

Apoptotic effects of AMD and DME

We investigated whether AMD and DME suppress cell growth in part by inducing cell apoptosis since the 5-day DME-treated cell number was lower than the day 0 control. Bcl-X_L, an anti-apoptotic factor [24], and Bax, a proapoptotic protein [25], were analyzed in AMD- and DME-treated cells. Treatment of LNCaP C-81 cells with AMD and DME caused time-dependent upregulation of Bax protein seen at day 3 and day 5 (Fig. 5C). At day 5, p53 protein, a tumor suppressor, was also elevated in both AMD- and DME-treated cells (Fig. 5C). In parallel, AMD and DME treatments caused a significant decrease of Bcl-X_L protein, compared to untreated control cells (Fig. 5C). These results together indicate that induction of apoptosis is one mechanism by which imidazopyridine derivatives induce growth suppression in PCa cells under steroid-reduced conditions.

Effect of AMD and DME on AR and PI3K/Akt signaling

Akt activation due to loss of PTEN activity can potentially support CR PCa formation [26–28]. In parallel, aberrant AR signaling plays a critical role in CR PCa progression [19,29,30]. Hence, it is proposed that the combined inhibition of AR and PI3K/Akt can effectively improve the therapeutic efficacy in CR PCa patients. We determined whether AMD and DME can inhibit PI3K/Akt by examining S473 phosphorylation of Akt in LNCaP C-81 cells in which Akt is fully activated by S473 phosphorylation [16]. We used PI3K and Akt inhibitors for comparison. Fig. 6 showed that AMD and DME treatments decreased the S473-phosphorylation of Akt in addition to decreased AR and PSA proteins in LNCaP C-81 cells (Figs. 5B and 6). As expected, both PI3K and Akt inhibitors respectively reduced Akt(S473) phosphorylation (Fig. 6). Interestingly, Akt inhibition greatly increased cellular prostatic acid phosphatase (cPAcP) level, a tumor suppressor gene in PCa [31], but not PI3K inhibition (Fig. 6). Unexpectedly, AR protein level was respectively elevated following PI3K and Akt inhibitor treatments (Fig. 6). The elevated AR expression level was corroborated by the increased cellular PSA level.

Discussion

In androgen-dependent cells, upon androgen binding, AR is activated and then translocated to nucleus for gene regulation, resulting in cell growth and survival [29,30,32]. Nevertheless, CR PCa cells, which still require functional AR, evade ligand-dependent and ligand-independent mechanisms that allow cells to survive in an androgen-depleted environment [14,29,30,32–34]. Thus, targeting multi-functional molecules simultaneously can improve the efficacy of therapy.

In the present study, LNCaP C-81 cells were used as the primary cell model for our studies because these cells exhibit many properties of CR PCa [12–14,33]. We first demonstrated the antiproliferative efficacy of three novel imidazopyridine derivatives on LNCaP C-81 cells (Fig. 2). Among them, AMD effectively inhibits the cell growth evidenced by decreased cell number and cyclin protein levels (Fig. 2). Further, Bax and p53, a Bax transcription factor [34], are upregulated in AMD-treated cells. Hence, AMD inhibits cell proliferation. Importantly, while AMD has a broad spectrum of antiproliferative activity in both AR-positive and AR-negative AI PCa cells; AMD spares normal prostate epithelial cells (Fig. 2E).

To improve the efficacy of growth suppression on AI PCa cells, we modified the amino group side chain of AMD and obtained a derivative termed DME (Fig. 1). DME exhibits superior inhibitory activity compared to AMD on LNCaP C-81 cell growth in regular as well as SR media (Fig. 3). While both AMD and DME effectively inhibit LNCaP C-81 and PC-3 cell proliferation (Figs. 2, 3 and 5), these two compounds may exhibit different mechanisms of inhibition towards these two cell lines. For example, in the wound healing assay, AMD was a more potent inhibitor of PC-3 cell migration than DME. Unfortunately, LNCaP C-81 cells migrate too slowly to conduct a wound healing assay. Conversely, in both clonogenic and soft agar assays conducted on LNCaP C-81 cells, DME was a more potent inhibitor of colony formation than AMD (Fig. 4). One possible explanation of differential effects by DME vs. AMD is that DME is more potent than AMD at inhibiting AR protein level and Akt activation in C-81 cells. Additionally, PC-3 cells are

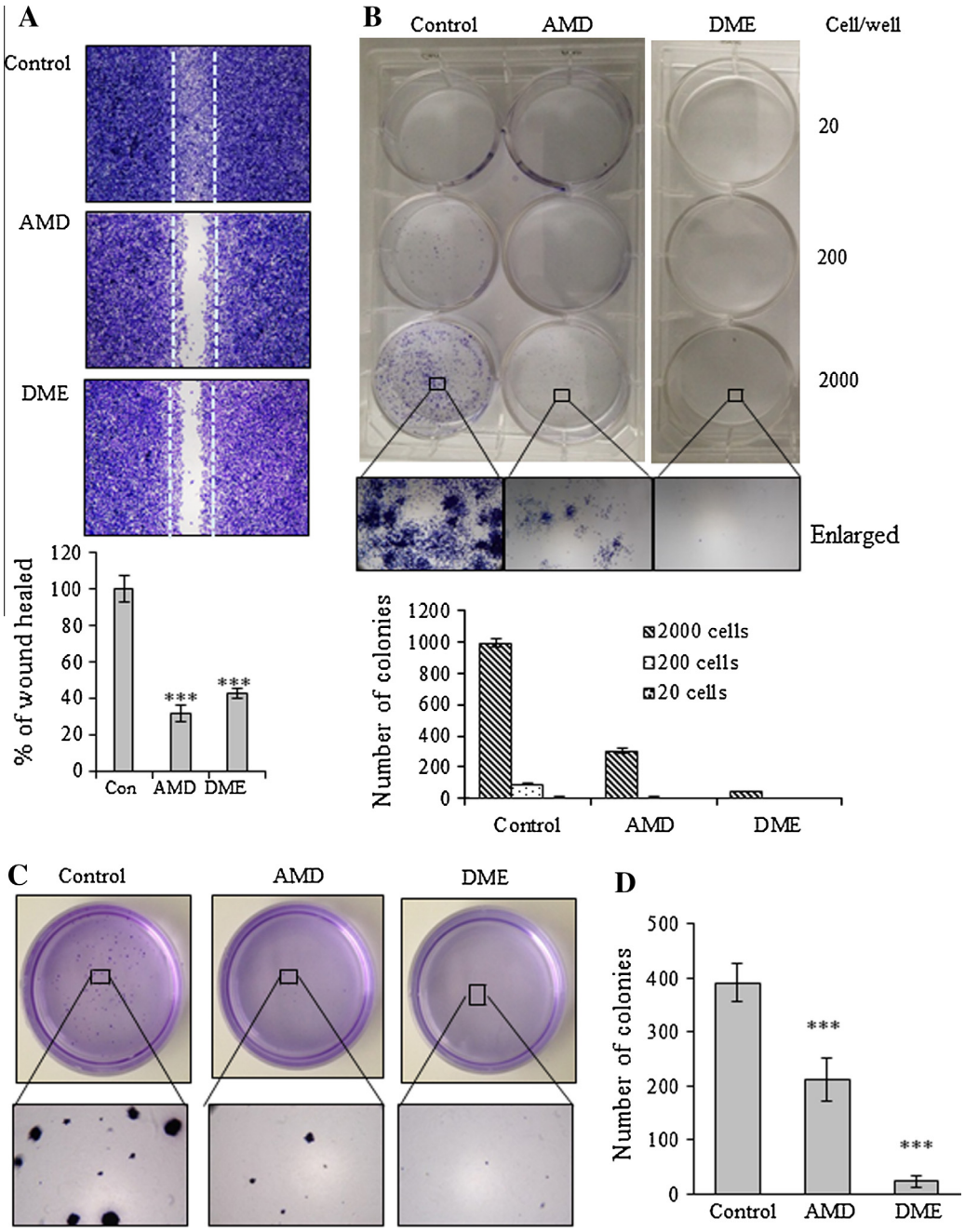


Fig. 4. Effects of AMD and DME on PCa cell migration and tumorigenicity. A. PC-3 cells were seeded in a 6-well culture plate (1×10^4 cells/cm^2) until 90% confluence. A wound was created on monolayer culture using 200 μl pipette tip, and the cells were then treated with or without 10 μM AMD or DME for 12 h. At the end of the experiment, cells were stained, photographed and the migration inhibitory-potential of AMD and DME was determined by measuring the wound closure. $n = 2 \times 4$, $^{***}p < 0.001$. B. LNCaP C-81 cells were plated in plastic wares at the densities of 20, 200 and 2000 cells/well. After 24 h the attached cells were treated with respective compounds at 10 μM concentration of AMD and DME. The cells were fed on days 5 and 8 with fresh culture media with respective inhibitors. On day 10, the cells were stained and photographed. The bottom picture was the enlarged version under microscope and the colony number was counted. C. LNCaP C-81 cells were plated at the densities of 5×10^4 cells/35 mm dish in soft agar plates. At the end of 4 weeks, the colonies formed were stained and that from single cell was counted. Representative images of colony formation were shown. The lower panel is microscope enlarged images. D. Number of soft agar colonies in respective groups. The results presented were mean ± SE; $n = 2 \times 4$. $^{***}p < 0.001$.

AR-negative and LNCaP C-81 cells express functional AR; as such, DME and AMD inhibit both AR and Akt in LNCaP C-81 cells, while only inhibit Akt in PC-3 cells. Together, DME is more potent than AMD at inhibiting LNCaP C-81 cell proliferation and colony formation. Nevertheless, further experiments with direct comparison are required to delineate the mechanism of growth suppression by DME vs. AMD on these two cell lines.

Targeting AR and/or intervening with androgen biosynthesis can effectively inhibit CR PCa and thus improve patient survival [35–38]. Our results show for the first time that both AMD and DME treatments can decrease AR protein and signaling with cell growth suppression (Figs. 2, 5A and B). Although the mechanism of AR inhibition by imidazopyridine derivatives requires further investigation, our results show that imidazopyridine derivatives inhibit AR-positive PCa cell growth in part by inhibiting AR signaling, evidenced by decreased PSA protein (Figs. 5B and 6). It is possible that imidazopyridine derivatives decrease AR protein partly by inhibiting Akt phosphorylation. AMD and DME inhibit Akt-induced AR protein stability, contributing to observed lower levels of AR protein. Furthermore, by inhibiting AR signaling, AMD and

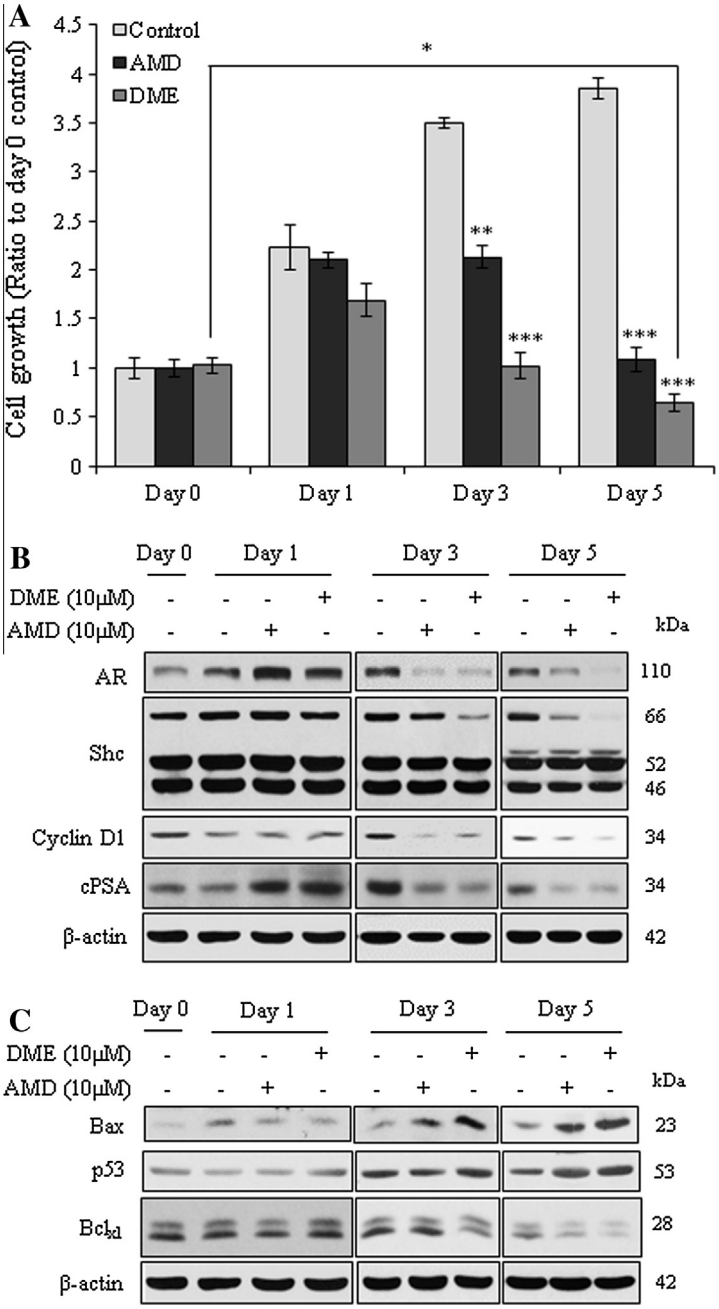


Fig. 5. The kinetic effects of AMD and DME on LNCaP C-81 cells in steroid-reduced conditions. A. The cells were plated in six-well plates at 2×10^3 cells/cm^2 in regular medium for 3 days, then steroid starved for 48 h followed by treatment with AMD and DME at the concentration of 10 μM. After 1, 3 and 5 days, one set of cells (in duplicates) from each group was harvested for cell number determination. The remaining cells were replenished with fresh medium and inhibitors (AMD and DME). B. Total cell lysate proteins from AMD- and DME-treated C-81 cells were analyzed for AR, Shc, PSA and cyclin D1 protein. β-Actin protein level was used as a loading control. C. The pro-apoptotic effect of AMD and DME on LNCaP C-81 cells in a steroid-reduced condition with time dependent manner. Total cell lysate proteins from AMD and DME treatment were analyzed for p53, Bcl-X_L and Bax proteins. β-Actin protein level was used as a loading control. The results presented were mean ± SE; $n = 2 \times 3$. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

DME may prevent the interaction of AR with p85α regulatory subunit of PI3K; which results in decreased Akt activation [39]. p66Shc, a 66 kDa oxidase, can up-regulate cell growth and is elevated in steroid-regulated carcinomas [20,23,40]. In PCa, p66Shc plays a role in mediating the cross-talk signal between steroids and tyrosine phosphorylation [40], which is involved in regulating cell proliferation and apoptosis [22,23,40,41]. Further, androgens upon binding to AR can up-regulate p66Shc protein level and PCa cell proliferation [40]. Our data show that both AMD- and DME-treatments resulted in decreased AR and p66Shc protein levels, correlating with diminished cell proliferation and elevated apoptotic Bax protein (Fig. 5). Although LNCaP C-81 PCa cells are androgen-independent cells, their growth is still increased upon androgen treatment [13,14,19]. Thus, imidazopyridine derivatives inhibition of AR-mediated cell growth and p66Shc protein levels is at least in part through decreased AR protein levels. Thus, p66Shc protein may serve as a common convergent point that integrates cell proliferation and apoptosis signaling [23].

CR PCa cells often have elevated levels of anti-apoptotic molecules for its survival [29,42]. Bcl-2 family members play critical roles in regulating apoptosis. For example, Bcl-X_L is anti-apoptosis [43,44] and can also enhance metastasis [45]; while Bax can

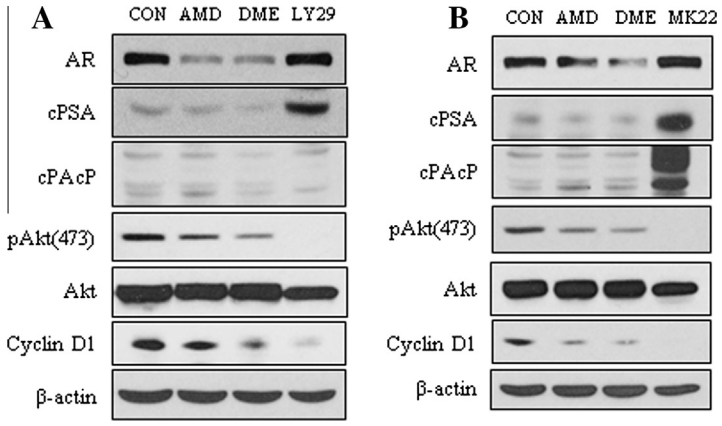


Fig. 6. Effects of AMD and DME on AR and PI3K/Akt signaling in LNCaP C-81 cells under steroid-reduced culture conditions. Cells were plated in T25 flasks at 4×10^3 cells/cm^2 in regular medium for 3 days, then steroid starved for 48 h followed by treatment with 10 μM AMD, DME, PI3K inhibitor (LY294002) (A) or pan Akt inhibitor (MK2206) (B). Control cells (Con) received solvent alone. After 72 h cells were harvested and analyzed for pAkt, AR, cPSA, cPAcP (cellular prostatic acid phosphatase, a PTEN-functional homologue in prostate epithelia, functions as a prostate-specific tumor suppressor) and cyclin D1 protein levels. β-actin protein level was used as a loading control.

heterodimerize with Bcl-X_L and its increased level favors apoptosis [25,46]. Further, p53 protein is a tumor suppressor, a Bax transcriptional regulator [34,47] and also mediates a Bcl-2 family member-independent apoptosis process. The elevated Bax and p53 with decreased Bcl-X_L proteins (Fig 5C) together indicate AMD and DME can induce apoptosis. Therefore, we observed DME- and AMD-induced growth suppression and DME-treated cells had cell number even lower than that of day 0 control cells (Fig. 5A).

The PI3K/Akt signaling pathway plays a vital role in cell growth and survival; its dysregulation contributes to therapeutic resistance of tumor cells [26,27,29,30,48]. Our results show AMD and DME inhibit Akt activation in addition to AR signaling (Figs. 5 and 6). Unexpectedly, treatments with both PI3K and Akt inhibitors induce AR and PSA levels in PTEN-inactive LNCaP C-81 cells (Fig. 6), which is consistent with the observation on the reciprocal activation of AR and PI3K signaling in PTEN-deficient mouse PCa cells [28]. Hence, the data indicate that patients treated with PI3K pathway inhibitors can experience a rise in PSA levels if their tumors are PTEN deficient [28]. Further, it is also hypothesized that Akt activation may inhibit HER-2-mediated AR activation [28]. Our results clearly show that these novel imidazopyridine derivatives significantly inhibit both AR and PI3K/Akt signaling. Even though targeting either AR or PI3K/AKT signaling alone by second generation inhibitors has shown promising results [28,49]; the activation of reciprocal pathways can counteract the efficacy of inhibitors targeting single signaling pathway in PCa [28,50]. Hence, combination therapy targeting both AR and PI3K/Akt signaling molecules would improve the therapeutic efficacy toward CR PCa.

In summary, our data show for the first time that imidazopyridine derivatives can effectively inhibit CR PCa cell proliferation in SR conditions and reduce *in vitro* tumorigenicity. The concurrent inhibition of PI3K/Akt and AR signaling with the activation of apoptotic pathway is one of the underlying mechanisms that imidazopyridine derivatives suppress CR PCa cell growth. Our data thus support the notion that the imidazopyridine derivatives represent a promising class of compounds to combat CR PCa. Further studies are needed to elucidate the underlying suppression mechanisms on CR PCa cells. Modifications of other functional groups of these compounds may further improve the efficacy of PCa suppression for treating CR PCa.

Conflict of Interest

We do not have any conflict of interest.

Acknowledgements

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2012 INBRE-CAU Scholars First Week Schedule

Michael Sorrell Center Room 4053, UNMC

Tuesday-May 29

8:00	Welcome and Introductions	J. Turpen P. Davis
9:00	Use of Animals in Research	T. Rosenquist
9:45	Laboratory Safety	W. Chaney
10:45	Introduction to Bioinformatics	H. Ali
12:00	Lunch	
1:00	Library Access	M. Helms
1:45	Responsible Conduct in Research	D. Crouse
2:30	Biotech Ideas to Products	M. Dixon
3:45	Radiation Safety Usage and Video	W. Chaney
4:45	Wrap-up and Questions	
5:30	Barbeque Welcome Banquet	J. Turpen

Wednesday-May 30

9:00	Science as a Career	D. Crouse
9:50	Graduate Studies at UNMC	D. Crouse
10:30	MD/PhD Program at UNMC	S. Smith
10:50	Graduate Studies at Creighton	R. Hallworth
11:20	Graduate Studies at UNL	J. Morris
12:00	Lunch	
	Go meet mentors and labs.	

2012 INBRE-CAU Weekly Seminar Schedule
(all on Mondays)

June	4	UNMC	9:00	C. Kuscysnki	Single Cell Flow Analysis
			10:30	Deb Romberger	Pulmonary Research at UNMC
June	11	UNL	9:15	Jack Morris	Morrison Center Introduction
			9:30	Clint Jones	Herpesvirus
			10:30	Shi-hua Xiang	HIV Vaccine Development
			11:15	Matt Wiebe	Poxviruses
June	18	CUMC	9:00	Shashank Dravid	Inotropic Glutamate Receptors
			10:00	S. Lovas, H. Smith	Tour of Proteomic and Confocal Microscopy Core Facilities
			11:00	S. Lovas	Structure-Activity Relationships in Polypeptides
June	25	Omaha Biotech	9:00	Terri Wasmoen Invertis-Merck	Vaccine Development
July	2	Lincoln Biotech	9:15	Susan Lynch	Welcome and Introductions
			9:30	Michelle Combs	Tour of the Celerion Clinical Pharmacology Sciences Operations
			10:30	Curtis Sheldon	Tour of the Bioanalytical Services
			11:30	Julie Saathoff	Q&A Session with Clinical Site Director
July	9	UNMC	9:00	J. Eudy	DNA Sequencing and Analysis
			10:30	K. Bayles	Infectious Disease Research at UNMC
July	16	UNL	9:15	Jack Morris	Beadle Center Introduction
			9:30	Tony Zera	
			10:30	Nicole Buan	
			11:00	Joe Zhou	Beadle Center/Microscopy Center Tours
July	23	CUMC	9:00	D. Yilmazer-Hanke	Role of Neuropeptide Y in Emotional Disorders and Epilepsy
			10:00	R. Hallworth	Scientific Writing Competition
			11:00	Lecture	TBD

May 27, 2012 – June 2, 2011 Week 0

		Sun 27	Mon 28	Tues 29	Wed 30	Thurs 31	Fri 1	Sat 2
7	:00 am						RAs/TAs arrive	Scholars Arrive by 5pm
	:30 am							
8	:00 am							
	:30 am							
9	:00 am							
	:30 am							
10	:00 am							
	:30 am							
11	:00 am	**Legend:**						
	:30 am	Travel						
12	:00 pm	Required, non-academic					Scholars Arrive after 12 noon	
	:30 pm	Required, academic						
01	:00 pm	Bus schedule – don't miss the bus!						
	:30 pm	Not required, just options for students to do on their own						
02	:00 pm	Not required, but we pay for some expenses if student go						
	:30 pm	Aksarben Village – walking distance from Scott Hall Overview: tinyurl.com/7ujsg2x map: tinyurl.com/7rwyza7						
03	:00 pm	Ideas of things to do in Omaha: tinyurl.com/75fozna visitomaha.com/things-to-do						
	:30 pm							
04	:00 pm							
	:30 pm							
05	:00 pm							Dinner and Orientation
	:30 pm							
06	:00 pm							
	:30 pm							

June 3, 2012 – June 9, 2011 Week 1

		Sun 3	Mon 4	Tues 5	Wed 6	Thurs 7	Fri 8	Sat 9
7	:00 am		Bus Leaves Scott Hall at 7:00 AM Floor 3					
	:30 am		Bus Leaves Scott Hall at 7:30 AM Floor 4					
8	:00 am		Orientation All M-F 8am-4pm in Sorrell 3002 unless otherwise noted	Assessments	Financial Workshop Wagoner	Biostatistics/Epidemiology Rohatgi All interaction rooms on Sorrell 3rd floor unless otherwise noted	Medical: Clinical, TAs Dental: Lincoln, Vogt	BLS Training Scott Hall Café
	:30 am							
9	:00 am							
	:30 am							
10	:00 am							
	:30 am		Opening Ceremony					
11	:00 am				Dr. Hill Admissions			
	:30 am							
12	:00 pm	Picnic and Name Badges						
	:30 pm		Compliance Training 12:30-1 Registration 1-3 Presentations					
01	:00 pm	Orientation		Financial Workshop Wagoner	Health Disparities: Johansson Interaction rooms 4th floor	Humanities Aita	Medical: Anatomy, Todd Sorrell 3002 and 1012 Dental: Lincoln	Community Service Projects
	:30 pm							
02	:00 pm							
	:30 pm							
03	:00 pm							
	:30 pm							
04	:00 pm		Bus Leaves UNMC at 4:15 PM Floor 3					
	:30 pm		Bus Leaves UNMC at 4:45 PM Floor 4				Bus and Van 4:45	
05	:00 pm	Opening BBQ						
	:30 pm							
06	:00 pm							VP Music tinyurl.com/cce7atz
	:30 pm							
			Book Club	Tempo of Twilight tinyurl.com/7wqzxpq	7PM Fireside Chat Ayman El-Mohandes		Arts Festival tinyurl.com/7fgzp5g	Arts Festival tinyurl.com/7fgzp5g

June 10, 2012 – June 16, 2011 Week 2

		Sun 10	Mon 11	Tues 12	Wed 13	Thurs 14	Fri 15	Sat 16
7	:00 am		Bus Leaves Scott Hall at 7:00 AM Floor 4					
	:30 am		Bus Leaves Scott Hall at 7:30 AM Floor 3					
8	:00 am		Biostatistics/Epidemiology Rohatgi	Biostatistics/Epidemiology Rohatgi	Biostatistics/Epidemiology Rohatgi	Biostatistics/Epidemiology Rohatgi	Medical: Clinical, TAs **Sorrell 3001** Dental: Lincoln, Vogt	
	:30 am							
9	:00 am	Community Service Projects						
	:30 am							
10	:00 am							
	:30 am							
11	:00 am							
	:30 am							
12	:00 pm							
	:30 pm							
01	:00 pm	Community Service Projects	Writing Skills Myers	Study Skills Peter	Health Disparities: Johansson **Interaction rooms 4th floor**	Humanities Aita	Medical: Clinical, Todd **Sorrell 3001 and 1012** Dental: Lincoln, Vogt	
	:30 pm							
02	:00 pm							Bus leaves 2pm and 6pm
	:30 pm							College World Series Games at 4pm and 8pm cwsomaha.com
03	:00 pm							
	:30 pm							
04	:00 pm		Bus Leaves UNMC at 4:15 PM Floor 4					
	:30 pm		Bus Leaves UNMC at 4:45 PM Floor 3				Bus and Van 4:45	
05	:00 pm							
	:30 pm					Bus – time?		
06	:00 pm	SAB Elections				CWS Opening Ceremony 8:30 pm cwsomaha.com	CWS 8pm cwsomaha.com	
	:30 pm							
			Book Club	Tempo of Twilight tinyurl.com/7wqzxpq	7pm Fireside Chat Amber Shelter Teri Hartman			Bus returns after games

June 17, 2012 – June 23, 2011 Week 3

		Sun 17	Mon 18	Tues 19	Wed 20	Thurs 21	Fri 22	Sat 23
7	:00 am		Bus Leaves Scott Hall at 7:00 AM Floor 3					
	:30 am		Bus Leaves Scott Hall at 7:30 AM Floor 4					
8	:00 am		Physics Sowell	Physics Sowell	Physics Sowell	Physics Sowell	Medical: Clinical, TAs **Sorrell 3002, but only after 10am** Dental: Lincoln, Vogt	
	:30 am							Bus leaves 8:30
9	:00 am							Huskers Stadium 8:30 am – 4 pm
	:30 am							
10	:00 am							
	:30 am							
11	:00 am							
	:30 am							
12	:00 pm							
	:30 pm							
01	:00 pm		Writing Skills Myers	Study Skills Peter	Health Disparities: Johansson **Interaction rooms 4th floor**	Humanities Aita	Medical: Anatomy, Todd Dental: Lincoln, Vogt	
	:30 pm							
02	:00 pm							
	:30 pm							
03	:00 pm							Bus leaves after event
	:30 pm							
04	:00 pm		Bus Leaves UNMC at 4:15 PM Floor 3					
	:30 pm		Bus Leaves UNMC at 4:45 PM Floor 4				Bus and Van 4:45	
05	:00 pm	RA Cookout and Talent Show						
	:30 pm							
06	:00 pm							VP Music tinyurl.com/cce7atz
	:30 pm							
			Book Club	Tempo of Twilight tinyurl.com/7wqzxpq	7pm Fireside Chat Devin Nichol Shelia Ryan	Shakespeare On the Green tinyurl.com/4y3o9sc	Shakespeare On the Green tinyurl.com/4y3o9sc	Shakespeare On the Green tinyurl.com/4y3o9sc

June 24, 2012 – June 30, 2011 Week 4

	Sun 24	Mon 25	Tues 26	Wed 27	Thurs 28	Fri 29	Sat 30
7 :00 am		Bus Leaves Scott Hall at 7:00 AM Floor 4					
:30 am		Bus Leaves Scott Hall at 7:30 AM Floor 3					
8 :00 am		Physics: Sowell **Sorrell 3001**	Chemistry Sullivan **Sorrell 3001**	Chemistry: Sullivan	Chemistry Sullivan	Medical: Clinical, TAs Dental: Lincoln, Vogt	
:30 am							
9 :00 am							Bus?
:30 am							
10 :00 am							SAB Planned Event
:30 am							
11 :00 am							
:30 am				Evaluations Sorrell 1010			
12 :00 pm							
:30 pm							
01 :00 pm	Community Service Project	Writing Skills Myers **Sorrell 3001**	Hugh 10min	Health Disparities: Johansson	Humanities Aita	Medical: Anatomy, Todd Dental: Lincoln, Vogt	
:30 pm			Study Skills Peter **Sorrell 3001**				
02 :00 pm							
:30 pm							Bus?
03 :00 pm							
:30 pm							
04 :00 pm		Bus Leaves UNMC at 4:15 PM Floor 4					
:30 pm		Bus Leaves UNMC at 4:45 PM Floor 3				Bus and Van 4:45	
05 :00 pm							
:30 pm							Bus leaves Scott at 5:30
06 :00 pm						Memorial Park Concert tinyurl.com/2fyy2ad	7:05pm Omaha Storm Chasers
:30 pm							
	Shakespeare On the Green tinyurl.com/4y3o9sc	Book Club		7pm Fireside Chat Dub Vogt Dave Carver Patrick Tyrance, Jr	Shakespeare On the Green tinyurl.com/4y3o9sc	Shakespeare On the Green tinyurl.com/4y3o9sc	Bus leaves after game

July 1, 2012 – July 7, 2011 Week 5

	Sun 1	Mon 2	Tues 3	Wed 4	Thurs 5	Fri 6	Sat 7
7 :00 am		7:00 AM			Floor 3		
:30 am		7:30 AM			Floor 4		
8 :00 am		Chemistry Sullivan	Chemistry Sullivan		Biology Crouch	Medical: Clinical, TAs Dental: Lincoln, Vogt	
:30 am							
9 :00 am							
:30 am				Bus leaves Scott 9:30			Bus leaves Scott at 9:30
10 :00 am				July 4th Mahoney State Park			Zoo Trip 9:30 to 4 pm
:30 am							
11 :00 am							
:30 am							
12 :00 pm							
:30 pm							
01 :00 pm	Blood drive Scott Hall games planned by SAB	Writing Skills Myers	Study Skills Peter		Humanities Aita	Medical: Anatomy, Todd Dental: Lincoln, Vogt	
:30 pm							
02 :00 pm							
:30 pm							
03 :00 pm		Video Conference with UVA CON 1005					
:30 pm							
04 :00 pm		4:15 PM		Bus leaves Park 4pm	Floor 3		Bus leaves Zoo at 4pm
:30 pm		4:45 PM			Floor 4	Bus and Van 4:45	
05 :00 pm							
:30 pm							
06 :00 pm							Bus leaves Scott at 6pm
:30 pm				Bus needed time?			VP Music tinyurl.com/cce7atz
	Shakespeare On the Green tinyurl.com/4y3o9sc	Book Club	7pm Fireside Chat Renaisa Anthony Davies	Downtown Fireworks time?	Shakespeare On the Green tinyurl.com/4y3o9sc	Shakespeare On the Green tinyurl.com/4y3o9sc	Bus returns after event

July 8, 2012 – July 14, 2011 Week 6

	Sun 8	Mon 9	Tues 10	Wed 11	Thurs 12	Fri 13	Sat 14
7 :00 am		Bus Leaves Scott Hall at 7:00 AM Floor 4					Scholars Depart by 12 noon
:30 am		Bus Leaves Scott Hall at 7:30 AM Floor 3					
8 :00 am		Biology Crouch	Biology Crouch	Biology Crouch	Biology Crouch	Medical: Clinical, TAs Dental: Lincoln, Vogt Dental Back by 12 noon	
:30 am							
9 :00 am							
:30 am							
10 :00 am							
:30 am							
11 :00 am				Evaluations **Sorrell 1012**			
:30 am							
12 :00 pm							
:30 pm							
01 :00 pm		Writing Skills Myers	Study Skills Peter	Health Disparities: Johansson **Interaction rooms 4th floor**	Humanities Aita		
:30 pm	Bus?						
02 :00 pm	Boystown Tour 2-4					Closing Ceremony	
:30 pm							
03 :00 pm							
:30 pm						Bus and Van 3:30	
04 :00 pm		Bus Leaves UNMC at 4:15 PM Floor 4					
:30 pm		Bus Leaves UNMC at 4:45 PM Floor 3					
05 :00 pm	Bus?						
:30 pm						Scholars Depart after 5pm	
06 :00 pm				Banquet and Dance 6-11			
:30 pm							
	Shakespeare On the Green tinyurl.com/4y3o9sc	Book Club	7pm Fireside Chat John Reinhardt Mariann Fossum		Jazz on the Green tinyurl.com/29cl4km		

Mentors and Research Projects

Investigator	**Institution**	**Project**
S. Batra	UNMC	Genetic Alterations in Prostate Cancer Progression and Experimental Therapy
W. Chaney	UNMC	Glycobiology in Prostate Cancer
P. Cheng	UNMC	Glycomics in Prostate Cancer and Metastasis and Gene Therapy
K. Datta	UNMC	Redox in Prostate Cancer Metastasis and Therapy
J. Davis	UNMC	Hormone Regulation of Tumor Cell Development
R. Lewis	UNMC	IGF Receptor signaling in Cancer Progression and Experimental Therapy
M.-F. Lin	UNMC	Androgen Regulation of Prostate Cancer Growth and Progression and Experimental Therapy
P. Mehta	UNMC	Gap Junction Proteins in Prostate Cancer Metastasis
J.L. Mott	UNMC	microRNA in Cancer Apoptosis
M. Simpson	UNL	Role of Hylauronate in Prostate Cancer Development and Metastasis
Y. Tu	CU	G-Protein-Coupled Receptors in Prostate Cancer Metastasis and Therapy